STERNAL PUNCTURE

A METHOD OF CLINICAL AND CYTOLOGICAL INVESTIGATION

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FOREWORD

STERNAL PUNCTURE is the generally accepted means by which myeloid tissue may be studied during life. It is one of the most modern of the techniques included under the term "clinical pathology." A little hesitant at first, it has now developed into a full-blown method of investigation.

By the help of sternal puncture three things are being accom-

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states of the body are being observed. All these aspects of the subjects are covered in Dr Piney's present monograph.

The limits of usefulness of the new technique cannot yet be stated. They must await much more exploitation of the method than has so far been undertaken.

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the liver occur as possible examples.

The authors have avoided dogmatism and have wisely kept such knowledge as we have in this field in a fluid state, where it should be allowed to remain whilst the technique is in its early history and the body of ascertained facts relatively small. Equal care has been shown in handling the nomenclature.

The book is timely because it summarises the present position of the subject and acts as a manual for investigators, whether their approach is on the pathological or on the clinical side.

The popularity which has been achieved by Dr Piney's previous books in hæmatology may safely be predicted for this new work also.

HORDER

PREFACE TO FOURTH EDITION

THE need for a fourth edition of this book suggests that the mode of presentation meets a real need, and we have, therefore, not altered the style by attempting to produce a complete review of the subject. There are many excellent text-books of hæmatology, some of which proceed from the consideration of the peripheral blood to a discussion of the marrow, while there are others in which the opposite course is adopted, but our aim has been (and still is) to present the essential features of the bone-marrow in health and disease. For this reason, we have not increased the size of the book, but have extended our consideration of the relevant literature, calling attention to such papers as contain an adequate bibliography.

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CONTENTS

LIST OF COLOUR PLATES

INTRODUCTION

Blood examination, which has become an important adjuvant to both diagnosis and prognosis has always suffered from the weakness of depending for its value on the inferences drawn from it Knowledge of the concomitant changes in the formative tissues has been scanty Attempts have often been made to correlate blood changes with those in the marrow, but the difficulties have been great First, the blood has been examined by the film method, whereas the marrow has been examined in sections when the cells look very different Secondly, marrow sections can usually only be obtained from post mortem material and will then reveal only the terminal state

Marrow puncture is a convenient method of examining the formative tissue during life, and it can, without difficulty, be performed several times on the same patient There can be no doubt that it has led to a much deeper knowledge of the factors on which the characters of the blood picture depend

The blood is, of course, not a tissue in the usual sense of that word it is a product of a number of organs of which the bone marrow is the most important, and yet the blood is not a secretion of the hæmopoietic tissue in the same sense that urine is a secretion of the kidneys It does not consist of constituents that have been abstracted from the body as a whole but of portions of the parent tissue itself It is for this reason that it is convenient to conceive of the whole mass of circulating cells together with those in the formative tissues as forming an organ Thus Boycott spoke of the *erythron* as the organ composed of the whole collection of red cells and their precursors in the body A similar concept, the *leucon*, is applicable to the white cells, and the *thrombon* to the platelets

These organs are not totally independent of one another because the primitive parent cells of both the red and the white elements are probably identical, in other words, at the earliest stage, the erythron, the leucon and the thrombon meet For this reason we must make use of the conception of the *hæmaton*, which is then the whole of the blood and the blood forming system Thus, examination of the marrow is so important because it reveals something more than the characters of the parent tissue of the blood, it shows us an integral part of the hæmaton

It was the improper abstractive separation of the circulating blood from the formative tissues that led in the past to a deep and fundamental cleavage in the hæmatological world Thus, there were two main schools of thought—the monophyletic and the polyphyletic The former contended that all the different types of blood cells were derived from a common ancestral form, which was variously known as the lymphoidocyte, the hæmocytoblast, and about eighty other synonyms The polyphyletists, on the other hand, asserted that there were several stem cells, each one with irreversibly determined potentialities The number of such elements was a matter of discussion among the several groups of this school, but all were agreed that the monophyletists were wrong

This controversy, which, in the past, filled thousands of pages, is no longer acute because our knowledge of the marrow has increased In the past, most observations on the blood-forming organs were carried out by professional histologists, who naturally made use of their customary technique, that is to say, fixation, embedding in paraffin or celloidin, section cutting and staining, and most, if not all the histologists were adherents of the monophyletic view The clinical hæmatological workers, on the other hand, based their conclusions on observations made on stained films, and very many of them were polyphyletists

The divergence of views was to a very great extent dependent upon these differences of technique, because cells which appear large and full of cytoplasmic and nuclear detail, when seen in films, are small and difficult to recognise in fixed sections Marrow puncture has enabled us to use the same technique for marrow tissue as for blood, and, as a result, the old controversy has almost died down,

Various methods of obtaining marrow during life were used in the past Thus the femur was trephined and marrow extracted, or the tibia was similarly treated, and from both these methods a good deal of information was obtained There are two main objections to such procedures They are in the nature of major surgical operations and cannot be repeated frequently on the same patient Secondly, in adult life the red marrow in the limbs is confined to small areas at the upper ends of the humeri and femora, the more distal parts of these bones and the more distal bones of the limbs containing only fat In diseases, in which there is excessive production of cells, formative marrow may spread into all the bones Thus,

in advanced leukæmia and pernicious anæmia, valuable information could be obtained from the tibial bone-marrow, but *in health* and more acute maladies only fatty tissue was present. Obviously then, it would be a great advantage to obtain the specimen from a bone that is always filled with active marrow, such as the sternum. Since 1929, when Arinkin introduced the method of sternal puncture, this has been the usual technique.

It may be mentioned that marrow puncture is not only of value in the investigation of most of the disorders of the blood and in many diseases due to protozoa: it has been found that it may assist in the bacteriological diagnosis of typhoid fever. Rogge obtained a positive culture from the marrow in 60 per cent. of cases; and, in 9 cases, was successful in this way when all other attempts at culture had been unsuccessful. In the first week of the disease, 4 cultures out of 4 were positive, while only 19 out of 30 blood cultures were so. In the second week, 15 out of 21 sternal cultures were positive, against 9 out of 38 blood cultures. Thus, it would seem that typhoid fever cannot be excluded if the sternal marrow has not been cultured, even although all other tests have proved negative.

The material obtained by marrow puncture has even been used therapeutically. Morrison and Samwick claimed good results from intra-sternal injection of marrow juice in two cases of aplastic anæmia, but others have failed to obtain similar results. Schretzenmayr injected sternal marrow intramuscularly as a treatment for various anæmias, and believed that the method was of value: the evidence presented is, however, poor.

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STERNAL PUNCTURE

CHAPTER I

THE MYELOGRAM

THE recognition of the various types of cells in blood films needs a good deal of practice, and it is quite impossible to interpret the appearances in marrow films without a sound knowledge of the cytology of the peripheral blood This is, of course not surprising, because the marrow cells are either blood cells or are their precursors, and, for this reason we can use a similar terminology to that used in ordinary hæmatology

Schilling coined the name *hæmogram*, to designate the qualitative blood picture, and this is a much sounder concept than is the ordinary term "differential count" The term, *myelogram*, can with equal reason be applied to the total marrow picture Just as experience has given us knowledge of the normal percentages of the different types of cells in the blood in health and in a great variety of diseases, so observation has led to a knowledge of the marrow picture, the myelogram in health and disease

The hæmogram is naturally not an absolutely accurate picture of the cellular composition of the peripheral blood, because it is based on random sampling, both of the whole blood and even of the specimen taken for examination The myelogram is subject to the same sources of error, but is also less accurate than the hæmogram for another reason a varying proportion of the cells in the marrow cannot be classified either because of pathological changes in their structure, or, even in health by being in a more or less dedifferentiated state, prior to during or immediately after mitosis Even so, the myelogram is of great value, but has to be recorded in a more detailed manner than the hæmogram, because there are more classes of cells in the marrow than in the blood Then again, another difficulty is that marrow films cannot be evenly spread, there is admixture of a certain amount of fat and the cells are not floating free, as in blood but are more or less aggregated into clumps

Both for purely scientific and for clinical purposes, the myelogram and the hæmogram should be investigated at approximately the same time, and completeness would, of course, require examination of puncture fluid from the other hæmopoietic organs, spleen and lym-

more than describe its structure The cytoplasm, which is devoid of granules, is distinctly basophilic, and varies considerably in amount The nucleus is a good deal denser than that of the hæmohistioblast, but has the same type of reticular structure, being composed of interlacing fibrils of basichromatin, in which one or more nucleoli can easily be seen (Plate 3)

The followers of Ferrata regard this element as being the most primitive cell that has become irreversibly fixed in the direction of hæmopoiesis unlike its alleged ancestor, the hæmohistioblast, it can no longer produce connective tissue If this be so, the hæmocytoblast is the real stem-cell of the whole hæmopoietic process Certainly, in marrow that is hyperplastic from any cause, these cells are increased in number Thus, in the megaloblastic marrow of pernicious anæmia, in ordinary normoblastic marrow hyperplasia, in leucoblastic, and even in leukæmic marrow they are increased in number This fact seems to justify the inference that they are fixed in their general hæmopoietic potency, but are still multi potent, inasmuch as they can give rise to either red or white cells

On the other hand, Naegeli contended that the hæmocytoblast is not a multipotent stem cell, but that it is identical with the myeloblast the parent of the granular leucocytes only But his view fails to supply any explanation of the increase of these cells in all types of *hyperplastic marrow, if he were right, it would be surprising to* find these leucopoietic elements increased in erythroblastic reactions

As long as we are clear in our minds about the structure of these elements, so that we can recognise them, their exact status can be left to further research, but the name, hæmocytoblast, is now so widely used that we may well adopt it

Myeloblast The supporters of the view that the hæmocytoblast is multipotent, distinguish it from the myeloblast (Plate 2), which they consider to be an element irreversibly determined in the direction of granulocyte formation, that is to say, they regard it as a more differentiated cell It is, unfortunately, difficult to decide on what morphological criteria myeloblasts are to be distinguished from hæmocytoblasts It is said that the nuclei of the former are rather less tenuous, and that the cytoplasm is less basophilic But it is, of course, not to be expected that exact distinctions can be made, because we are dealing with a continuous process of development, not with isolated cell types

Premyelocyte *The premyelocyte (Plate 2) is intermediate* between the myeloblast and the myelocyte, that is to say, there is

no doubt that it is definitely fixed in its potencies, and its structural characters lie, as it were, midway between the two types of cell There seem to be two grades of premyelocytes First there are those in which the nucleus is only a little denser than that of a myeloblast and still contains nucleoli, but the cytoplasm is less basophilic and contains azurophilic granules Most of these cells have minute granules all of the same size (*preneutrophilic premyelocytes*) Some have large rather scanty granules, also all of the same size (*pre-eosinophilic premyelocyte*), and finally, there are a few elements with large granules of irregular size (*prebasophilic premyelocytes*) From these arise neutrophilic, eosinophilic and basophilic premyelocytes in which the nucleus has become a good deal denser and the nucleoli are obscured, while the granules have reached their definitive staining reactions

Myelocytes These cells are the stock from which most of the mature granulocytes arise in health In the marrow they vary greatly in size, some being little larger than lymphocytes, while a few are as much as 26 microns in diameter This variation in size is even greater than that seen in the blood in chronic myeloid leukæmia, although in the past it was supposed that the variations in that disease were due to the morbid process The nucleus of the myelocyte is circular but the edge may be slightly indented as if by pressure of the granules that fill the cytoplasm The chromatin still has a reticular arrangement resembling that of the myeloblast, but condensations and small nodules can be seen in the skein The cytoplasm is slightly basophilic but is difficult to see because it is almost filled with granules, which, at this stage, have, of course, reached their definitive staining reactions (Plate 2) The eosinophil myelocytes present difficulty, because, although many of them contain mature eosinophil granules, others present a mixture of granules, some being basophilic and others eosinophilic

A variable number of such elements is always found in the marrow, but the exact steps in their origin from premyelocytes are unknown It is possible that some of the cells in the marrow, which contain only basophilic granules, are really the very earliest stage of eosinophils If this is so, it would account for the fact that the marrow always contains more basophils than one would expect from the appearances of the peripheral blood

Metamyelocytes These are intermediate between myelocytes and mature polymorphs and can be divided into juvenile forms, with very slight indentations of the nucleus, and staff forms in which

the nucleus is deeply indented, rather like a band (Plate 2) It is at the stage of the staff form in the marrow that abnormalities of structure occur in pernicious anæmia, some of the cells being as much as 30 microns in diameter

Polymorphonuclears As seen in films of normal marrow, these cells are identical with those found in the blood (Plate 2), but, even when the blood polymorphs appear normal, atypical forms may be seen in the marrow Thus, in chronic myeloid leukæmia, very large ones may be seen, and in pernicious anæmia equally gigantic forms, mainly neutrophilic, with most complicated nuclei, are common in untreated cases

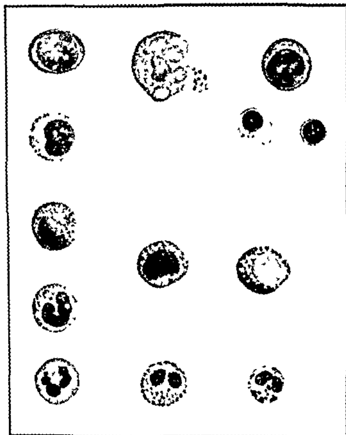
Lymphocytes These are not part of the myeloid tissue proper, *but there seems to be no doubt that the marrow is the seat of production* of a small proportion of the lymphocytes in the peripheral blood Certainly, *even in adults, it contains more large lymphocytes,* which are commonly supposed to be younger forms In rickets small aggregations of lymphocytes, sometimes even with a germ centre, may be found It is unusual to find immature cells of the lymphocyte series in normal marrow, but in glandular fever and in lymphatic leukæmia they may be numerous

The typical immature cell of this series is the *lymphoblast* (Plate 2), which is, so to speak, equal in its developmental potencies to the myeloblast, *i e* , it is irreversibly determined in the direction of lymphocyte formation It is about the same size as the myeloblast but with a relatively larger nucleus, which is more coarsely reticulated than that of the myeloblast It contains nucleoli around which the chromatin is somewhat condensed as it is also at the edge of the nucleus where it forms an almost *distinct membrane*

Monocytes Monocytes are scanty in normal marrow but appear *to arise from the hæmohistioblast through the stages of monoblast* and promonocyte The monoblast resembles the myeloblast and lymphoblast in general structure, and may not be easily distinguishable from them The promonocyte shows some indenting or folding of the nucleus with the characteristic ground glass cytoplasm of the adult monocyte , fine azurophilic granules are often visible in the cytoplasm The adult monocytes are in no way different from those seen in the blood

Plasma Cells. Plasma cells are scanty but invariably constituents of the marrow even in health They increase slightly in hypoplastic conditions and a great deal in myeloma There is still much controversy as to the origin *of these cells, and it remains un-*

PLATE 2



Development of White Cells

certain whether those of the blood and marrow are identical with those found in the tissues In the marrow most of these cells are quite large (15 to 20 microns), have an excentric nucleus, in which the basichromatin has a 'cart-wheel" arrangement, intense basophilic cytoplasm, and usually a pale area round the nucleus Similar cells, in which the nucleus is in the middle (Turk cells), are also found, and, like the plasma cells, their origin is uncertain

There is a good deal of evidence that any type of non granular blood or marrow cell may, for reasons as yet unknown, assume plasmacytoid characters so that it is probable that lymphocytic, lymphoblastic, myeloblastic and even erythroblastic plasma and Turk cells exist It has been asserted that plasma cells have a distinct line of development from plasmoblasts, and Moeschlin's work on these cells in rubella seems to support a similar view, although he gives no criteria by which one can distinguish plasmo blasts from lymphoblasts

Megakaryocytes Megakaryocytes are important components of the marrow and, in spite of their great size and irregular shape, they, or parts of them, are usually recognisable in films As they are rarely numerous, it is best to seek for them with low magnifications, and they are most likely to be found intact only near the edges and ends of films They are from 30 to 90 microns in diameter with faintly basophilic cytoplasm in which small groups of azurophilic granules are present, mainly near the edges (Plate 2)

The nuclei present most complicated lobulation, but there does not seem to be any distinctive distribution of the basichromatin, which is mainly disposed in the form of large masses It is possible that many of the structures, which in marrow films seem to be only parts of megakaryocytes, are really complete but immature elements which lie on the line of development from the histiocyte to the fully formed megakaryocyte The relative simplicity of the nuclei of these *megakaryoblasts* seems to support this view

There is much controversy about the nomenclature of the various megakaryocyte like cells that may be found in marrow films, but, of the many suggestions that have been put forward, those of Dameshek and Miller seem to have most to commend them

These authors distinguish the following forms —

(1) *Megakaryoblasts* which are about twice the size of myeloblasts, have non-granular cytoplasm, and a rather irregular single nucleus which contains several nucleoli

(2) *Promegakaryocytes* are about the same size as megakaryoblasts,

although much larger forms also occur The nucleus is usually not fully lobulated. The cytoplasm is rather scanty and is distinctly basophilic In it there are usually a few aggregations of azurophilic granules, resembling platelets, near the periphery of the cell body There may also be a few of these cells with *non-granular cytoplasmic* projections and such promegakaryocytes seem to be specially common in thrombocytopenic purpura

(3) *Lymphoid megakaryocytes* (rather a poor name) are large cells with relatively small lobulated nuclei and non-granular cytoplasm

(4) *Megakaryocytes*

(5) *Prepolykaryocytes* are mononuclear cells, which are often seen in small clusters The basophilic cytoplasm is vacuolated, and there is a reticulated nucleus which contains nucleoli

(6) *Polykaryocytes* are assumed to be fused syncytia of prepolykaryocytes , and are supposed to be osteoclasts under another name It is suggested that these cells may, by nuclear fusion, give rise to megakaryocytes an improbable occurrence

Red Cells It is now almost universally assumed that all red cells, both normoblasts and megaloblasts, arise from a common ancestor—the hæmocytoblast We need not enter into the time-honoured discussion of the genealogical history of the red cells in general, but, as the morphological differences between normoblasts and megaloblasts are considerable, they are best described separately

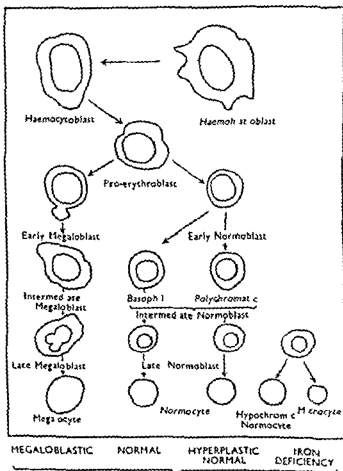
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of the ordinary red corpuscle In marrow films it is relatively easy to trace out every stage in this process

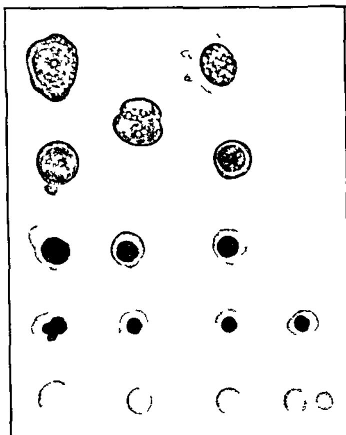
The least mature element that is recognisable as definitely belonging to the red cell series is the *pro erythroblast*, which is a large cell slightly resembling the hæmocytoblast but possessing a distinctive nuclear arrangement

The basichromatin is no longer purely reticular, as in the hæmocytoblast, and shows a tendency to be aggregated into almost triangular masses with a roughly radial arrangement Pale areas, which are perhaps nucleoli, can be detected, but the cells in which these can be seen are scanty The cytoplasm has become less intensely basophilic, and when no trace of nucleoli can be seen we have reached the next clear-cut stage—*the basophilic erythroblast.* In this the radial arrangement of the chromatin is quite distinct, and



ERYTHROPOIESIS

PLATE 3



Erythropoiesis

of the different names used by different authors for the same cells In order to lessen this confusion we have constructed the accompanying table where an attempt has been made to correlate the different names used to describe the various cells, together with the authors who have used these names (p 11)

Atypical Erythropoiesis. Limarzi and Levinson have recorded an extremely bizarre and previously undescribed type of erythropoiesis observed in the bone-marrow of a man aged *seventy-seven suffering from* prostatic obstruction Very large cells (apparently giant basophil megaloblasts) were seen which underwent repeated mitotic division of the nucleus producing a multinucleated cell which eventually split to form ordinary basophil normoblasts, these developed into ordinary basophil normoblasts which in turn developed into ordinary erythrocytes Other similar cells behaved in the same way except that nuclear division occurred amitotically Where division of the nucleus was incomplete large nucleated red cells were formed which, after extrusion of the nucleus, left corpuscles as large as 23μ in diameter—these corpuscles did not appear in the peripheral blood but underwent a process of fragmentation in the marrow

Since this first report, Schwarz has studied many marrow films and found cellular gigantism and pluripolar mitosis of both the myeloid and erythroid cells This apparently may occur in many conditions as well as in normal people *Schwarz's paper does not* give the frequency with which this phenomenon occurs, but it must be comparatively rare as neither of us have ever seen it. Fig I, re drawn *after Schwarz, shows the types of cell which* may be encountered The changes apparently are not of pathological significance and the resulting monsters should be regarded as "sports"

The Normal Myelogram. It is not possible to make accurate total cell counts on the bone-marrow Segerdahl, who tried to do so, *found that in health the number varied from* 10,000 to 190,000 per cubic millimetre There can be little doubt that various factors *influence the total count* quite apart from the structure of the marrow *itself Thus, if only small amounts of marrow* are withdrawn by sternal puncture, the total number of cells per unit volume is greater than if a large amount is withdrawn, and, even more strangely, the *proportion of white cells is higher* There seems no doubt that it is only the differential count from which inferences can be drawn, and it is disappointing to find that the marrow obtained by sternal

FIG I

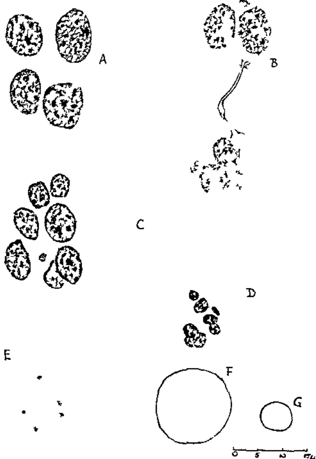


TABLE I *A correlation of the different names used by authors for various blood and stem cells The description of each cell will be found on the page indicated in the left-hand column*

This Book	Alternative Name	Author
Hæmohistioblast (p 3)	—	Ferrata
	—	Piney
Hæmocytoblast (p 3)	—	Schulten
	—	Israels
	—	Maximow
	Lymphoidocyte	Pappenheim
	Erythrogone	Helly
	Promegaloblast	Naegeli
	Megaloblast	Doan Cunningham and Sabin
Pro erythroblast (p 8)	—	Schulten
	—	Rinaldi and Ferrata
	—	Israels
	Erythrogone	Dameshek and Valentine
	—	Jaffé
	Promegaloblast	Nordensen
	—	Naegeli
	Megaloblast	Doan Cunningham and Sabin
	—	Peabody
Basophilic megaloblast (p 8)	Megaloblast	Doan Cunningham and Sabin
	—	Peabody
	Megaloblast A	Israels
Polychromatic megaloblast (p 9)	Megaloblast.	Ehrlich
	—	Turnbull
	—	Gilmour
	Megaloblast B	Israels
Eosinophil megaloblast (p 9)	Megaloblast C	Israels
Basophil normoblast (p 8)	Pronormoblast	Nordensen
	—	Naegeli
	Macronormoblast	Pappenheim
	—	Naegeli
	Early erythroblast	Doan Cunningham and Sabin
	Normoblast A	Israels
Polychromatic normoblast (p 8)	Late erythroblast	Doan Cunningham and Sabin
	Normoblast B	Israels
Eosinophil normoblast (p 8)	Normoblast	Doan Cunningham and Sabin
	Normoblast C	Israels

puncture before death gives a very different picture from that obtained by the same procedure within half an hour after death In other words, the inferences we may draw from the myelogram are much less certain than those which we may draw from the ordinary blood count

From the figures obtained by examining from 500 to 1,000 consecutive cells in marrow films, useful relationships can be revealed thus, in health, the proportion of white cells of all sorts to nucleated red cells varies from 5–1 to 3–1, but in the anæmias the proportion is much lower and the relationship may even be inverted Then again, the proportion of granular to non-granular white cells is of some importance Normally it is about 4–1 Again the numerical relationship of normoblasts to megaloblasts is significant, particularly in the diagnosis of pernicious anæmia

In the normal bone-marrow the cells multiply by ordinary karyokinetic division, and, in films of the marrow-fluid, mitotic figures are always seen It is important to arrive at some estimate of the percentage of dividing cells, and in which particular group of cells, *i e*, myeloblasts, myelocytes, etc, mitosis is most active, because from this information very useful deductions can be drawn Japa has given the following figures for normal bone marrow, in each 1,000 nucleated cells about 15 show mitotic figures, 40 per cent of these are in the prophase, 45 per cent in the metaphase, 10 per cent in the anaphase, and 5 per cent in the telophase The proportion of dividing leucoblasts to erythroblasts is given as 45 55, of myeloblasts to myelocytes 3 97, of early normoblasts to late normoblasts 91 9

In simple hyperplasia of the leucoblastic or erythroblastic tissues following acute pyogenic infection or acute hæmorrhage, the number of mitotic figures may double itself, but there will be no significant alteration in the ratios of the dividing cells in the myeloid and erythroid groups respectively The leukæmias, however, show a very different picture Here the number of dividing cells also shows a marked increase, but a larger percentage of the mitotic figures are in the more immature cells This is well seen in the myelogenous leukæmias, in the chronic forms there is an increase in the number of dividing premyelocytes, and to a much smaller degree in the myeloblasts as the malady becomes more acute the proportion of mitotic myeloblasts rises until, in the true acute form, they are the only dividing cells seen

NORMAL MYELOGRAM

Neutrophiles	
Myelocytes	30 –35 per cent
Metamyelocytes	10 –15 „ „
Polymorphs	24 –30 „ „
Eosinophiles	
Myelocytes	1 – 2 „ „
Polymorphs	½– 1 „ „
Basophiles	
Myelocytes	3 – 6 „ „
Polymorphs	1 – 2 „ „
Premyelocytes	1 – 2 „ „
Hæmocytoblasts, including myeloblasts	1 – 2 „ „
Hæmohistioblasts	¼– ½ „ „
Lymphocytes	
Small	6 – 9 „ „
Large	8 –12 „ „
Plasma cells	¼– 1 „ „
Pro erythroblasts	1 – 2 „ „
Normoblasts	15 –20 „ „
Megaloblasts	2 – 3 „ „
Megakaryocytes scanty, but invariable	

It is possible that some, if not all, the cells called megaloblasts in this table are really large normoblasts; but many writers state that a few genuine megaloblasts are present, even in health

Much has now been written on the variations in the normal myelogram Shapiro and Bassem have studied the sternal marrow changes during the first week of life and shown that there is a marked drop in the number of normoblastic cells at the end of the first week of life which runs parallel with the decrease in circulating reticulocytes Diwany gives the normal picture in children from one to five years of age, and Vogel and Bassem give the myelograms of forty-one children of various ages The hæmatology of the sternal marrow in normal women is discussed by Pitts and Packham, and the same authors and Markoff give the variations which are found during normal pregnancy (p 54)

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CHAPTER II

THE MARROW IN LEUKÆMIA

THE marrow changes in the various forms of leukæmia are usually distinctive, and for this reason it is best to start our description with this group of diseases It is not to be supposed that marrow puncture in the leukæmias is only of academic interest Admittedly, in many, perhaps most, cases of leukæmia, diagnosis is possible on clinical and ordinary hæmatological grounds Blood examination usually enables one to discover the type of leukæmia and also gives some indication of the severity of the condition, but the blood is not in the ordinary sense of the word a tissue it is a mixed secretion from the hæmopoietic organs, and for this reason the marrow naturally gives a clearer and more accurate picture than does the blood For instance, changes indicative of an approaching relapse will be found in the marrow earlier than in the blood, and, further, as all forms of treatment of leukæmia are directed towards the regulation of marrow function, it is of great value to follow the effects of therapy by repeated sternal punctures It should be recognised that whatever the improvement in the clinical condition the marrow is always recognisable as leukæmic Emile-Weil and Perles have demonstrated this quite clearly by regular sternal puncture during the treatment of a case of myeloid leukæmia The points to be looked for are variations in the relative percentages of the leukæmic cells, *e g*, the increase in the number of myeloblasts foreshadowing the change from chronic to acute myeloid leukæmia

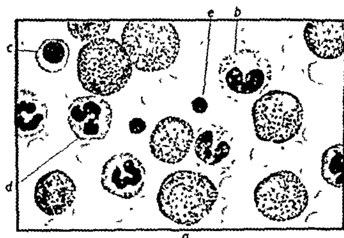
Chronic Myeloid Leukæmia (Chronic Leukæmic Myelosis) (Plate 4) The blood picture in this disease is usually striking and characteristic, but the changes in the marrow are less easily interpreted After all the marrow is the main organ in which granular leucocytes are formed, and it is extremely difficult to recognise slight degrees of overgrowth Fortunately, changes in the proportions of the cells occur early, and may enable one to make a diagnosis. Thus, in health, almost all the polymorphs arise from mitotic divisions of pre-existing myelocytes, myeloblasts are scanty and play little or no part in the production of fresh myelocytes In severe infections, where the demand for granular leucocytes is greatly increased, there may be so great a strain that the myeloblasts not only increase in number but also add to the stock of myelocytes

In chronic myeloid leukæmia, even in the stages when there is no increase in the number of cells in the blood, the myeloblasts take part *in the formation of myelocytes* , *in other words*, the whole process of granulopoiesis starts from a more primitive level If, therefore, an excess of myeloblasts is *found in marrow films, and if* infection can be excluded, a diagnosis of leukæmia is very probable When the leukæmic process is well established, the cellularity of the marrow is much increased, and this is so extreme that it can be recognised in well spread marrow-films where one may find as many as 80 cells in each field of the microscope In the ordinary chronic stage of the disease, this increase depends almost entirely upon the addition to the number of myelocytes, which may form as much as 80 per cent of the white cells Most of them are neutrophils, but excessive numbers of eosinophil and basophil myelocytes are also present Almost all the myelocytes of whatever type are larger *than the corresponding cells found in the blood, and many of them* are less mature It is particularly in the marrow that one can easily find myelocytes containing both eosinophilic and basophilic granules

Mitotic figures are not numerous in chronic cases, but it is very rare to be unable to find a few, usually in myelocytes and much more rarely in non-granular cells, which are presumably myeloblasts or hæmocytoblasts The number of myeloblasts and of premyelocytes is distinctly greater than in health, and some prognostic inferences can be drawn from the number of these immature elements The more plentiful *they are the worse the outlook In brief, it can be* said that any great increase in the number of cells, less differentiated *than myelocytes, is an indication* either that a relapse is imminent or that the disease is passing from the chronic into the acute stage This is, of course, comparable with what has long been known about the changes in the blood, viz that the greater the percentage of immature cells the more likely is an acute exacerbation, but the marrow changes precede those in the blood and therefore permit of earlier treatment On the other hand, undue alarm should not aroused by some myeloblasts in marrow films Even *if none* ‹ be found in the blood some will be seen in the marrow in all cases, and, *furthermore, they are always more* numerous in the marrow *than in the blood*

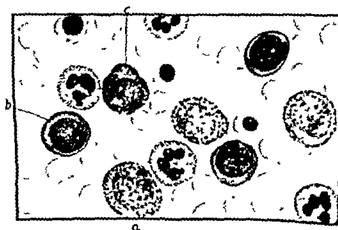
There are two other points of importance which are not easy to determine but which experience has shown to be of importance. The first is that the background of marrow films in chronic myeloid leukæmia is denser and contains more fragments of debris than in

PLATE 4



A CHRONIC MYELOID LEUKÆMIA

(a) Myelocyte (c) Basophile normoblast
(b) Metamyelocyte (d) Polymorph
(e) Normoblast



B CHRONIC MYELOID LEUKÆMIA BECOMING ACUTE

(a) Myelocyte (b) Myeloblast (c) Basophile Myelocyte

health Recognition of this change naturally depends upon experience of marrow films in general The second point, which is of great importance and which cannot be detected in films that are too thin or which are made from marrow fluid that is too dilute, is that the cells, or some of them, tend to lie in groups, in each of which there is only a single cell-type Thus, it is quite common in fairly thick films to find islets of myelocytes, premyelocytes and myeloblasts, indicating that in leukæmic marrow the immature cells have a more focal arrangement than normal It is in such islets that mitotic figures are most commonly seen If such figures are present in the myelocyte islets, the outlook is much less grave than it is when the myeloblasts show many mitoses

Marrow films in chronic myeloid leukæmia are the more pleomorphic because the leucoblastic overgrowth is accompanied by both erythroblastic and megakaryocytic reactions This, of course, introduces a difficulty in interpretation, because, unless the marrow is very carefully studied, the fact that every type of cell is increased might lead one to suppose that one was in the presence of a simple reactive hyperplasia such as may occur in association with any infection The recognition of the focal arrangement of the immature white cells and of the increase in the number of myeloblasts is the main safeguard against this false inference

It cannot be emphasised too strongly or too often that it is essential to correlate the blood picture with the myelogram , either alone can be most misleading For instance, there is no peculiarity in the bone marrow that would enable one to recognise the fact that the blood picture was aleukæmic The amount of hyperplasia is no greater in leukæmic cases than it is in aleukæmic ones, unless, of course, the aleukæmic state is due to treatment The factor that regulates emigration of cells from the marrow into the blood is unknown. At this point it may be well to mention that examination of the marrow is of particular importance in those cases in which the hæmogram has shown that the blood picture is aleukæmic Such a state may be due to partial aplasia of the marrow, either idiopathic or as the result of excessively energetic treatment , or it may be due to some unknown cause which prevents emigration of cells from the intensely active marrow into the blood It is obvious that in the former case any method of treatment such as X-rays will induce more rapid and more intense degeneration but, in the hyperplastic cases, irradiation of the marrow is as safe as in the ordinary leukæmic type of case

Moeschlin has compared the effects of urethane on the marrow

in chronic leukæmia with those of X-rays and arsenic He points out that the latter agents cause a diminution in the number of

the number of mitoses in the immature white-cells, while there is an increase in the number of dividing red cells Thus, urethane acts by a selective inhibition of mitosis in the essential " neoplastic " cells of the leukæmic process But the marrow does not show as great a decrease in the number of immature cells as it does after X-rays or arsenic

There are other cryptic forms of myeloid leukæmia in which

æmic or even n
in these cases
leukæmia but t
tenable because the blood picture and clinical condition deteriorate until death occurs Indeed, in prolonged cases, the medullary form of myelosis may develop into the ordinary type of chronic myeloid leukæmia Early diagnosis by marrow puncture is of importance because radiotherapy causes improvement even at a time when the spleen is still unaffected

There is also an acute form of this purely medullary overgrowth —*acute aleukæmic myelosis*—which clinically resembles the similar lymphatic condition

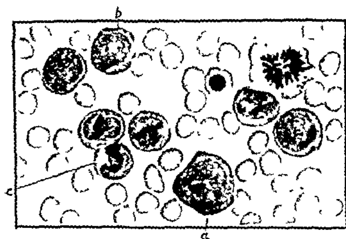
The relationship of *multiple myeloma* to *plasma-cell leukæmia* has not been clarified, but it is true to say that the more nearly plasma-cell leukæmia resembles typical leukæmia the more malignant is the process and the fewer are the nodular infiltrations

In the marrow it is, as a rule, not possible to distinguish plasma-cell leukæmia from plasma-cell myeloma, but, if films show a monotonous series of plasma cells unmixed with other elements, one is probably dealing with the latter condition The whole subject has been dealt with in some detail by Moss and Ackerman

The rare condition known as *eosinophile leukæmia*, which may run either an acute or a chronic course, presents varying appearances in the bone marrow

Acute cases usually end as myeloblastic leukæmia, but sometimes

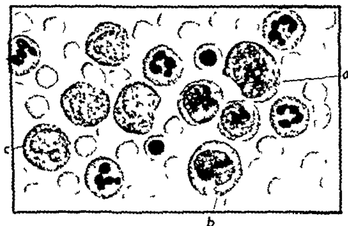
PLATE 5



A Monocytic Leukæmia

(a) Monoblast (b) Promonocyte

(c) Monocyte



B Monocytic Phase in Myeloid Leukæmia

(a) Monoblast (b) Monocyte

(c) Myelocyte

the predominant cell in the blood and in the bone marrow is the eosinophile myelocyte and this may be the case even in association with a very acute course In most of the chronic cases the majority of the eosinophiles in the circulation and in the bone marrow are *of the mature type so that it is impossible from either blood* or marrow examination to be certain whether the disease is chronic or acute

A somewhat similar malady may occur in an acute or more rarely a sub acute form—the so called *aleukæmic myelosis* This runs the clinical course of a rapidly progressive, severe anæmia without any change suggestive of leukæmia in the white cell count but the marrow has all the characters of the intensely active tissue found in ordinary cases of leukæmia Strangely enough one finds that the predominant cells in the marrow are myelocytes not as one would expect in an acute case myeloblasts If a case of this sort is encountered, it is extremely easy to draw false inferences concerning the acuteness of the disease if one is basing prognosis entirely on the characters of the myelogram The disease is associated with a variable degree of anæmia which is often macrocytic although it *may be normocytic or rarely, microcytic When a macrocytic* anæmia is present the marrow films will show a megaloblastic reaction and the picture simulates pernicious anæmia complicating leukæmia—the so-called leukanæmia of Leube Most modern writers regard the occurrence of these two diseases in one patient as fortuitous

Myelosclerosis may be very difficult to distinguish from medullary myelosis without the aid of sternal puncture, as the blood counts and physical signs may be almost identical in both diseases Most cases show fibrous tissue replacement of the marrow (which may lead to a dry puncture and the necessity for trephining *(p 81)) and no evidence of myeloid hyperplasia As in medullary* myelosis, the anæmia may be macrocytic, normocytic or microcytic, and the marrow will show the corresponding changes, in the last two types there are usually large numbers, up to 50 per cent of basophilic and polychromatic normoblasts

Chronic Lymphatic Leukæmia (Chronic Lymphadenosis) The myelogram in this disease is usually easy to interpret because lymphocytes are not numerous in normal marrow and rarely show any signs of mitotic activity In advanced cases of chronic lymphatic leukæmia, marrow films are as monotonous as *blood films Lymphocytes* are found so closely packed together that they almost resemble

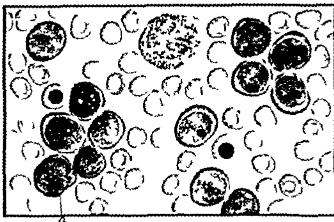
lymphatic tissue which is interrupted here and there by a few small nests of granulocytes and red cells The background of the marrow films is moderately dense but structureless and unlike that seen in chronic myeloid leukæmia where free cell granules are embedded in it In almost every case the majority of the cells are small lymphocytes, but a variable number of larger forms can be found not all of these are lymphoblasts, which, indeed, are usually scanty They increase in number only if the disease is actively progressing or is about to become acute

In less advanced cases of chronic lymphatic leukæmia, the lympho cytes can be seen to form small focal masses embedded in hyper-plastic marrow tissue In other words, the arrangement is similar to that in chronic myeloid leukæmia where we have already described foci of myeloblasts, etc Thus, lymphocyte aggregations in the marrow never possess germ centres, and indeed, it is mainly at their *periphery that the less immature forms* are seen, and mitotic figures are found

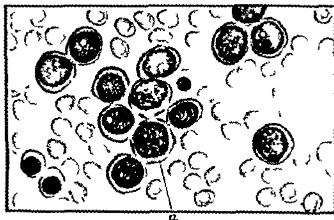
Chronic lymphatic leukæmia is notorious for its tendency to run an atypical course Most cases of so called crypto leukæmia are of lymphatic type

Chronic Aleukæmic Lymphatic Leukæmia This disease *may run* the whole of its course without any of the classical clinical signs of leukæmia Quite frequently the picture is that of aplastic anæmia—great reduction of red corpuscles and of hæmoglobin, no signs of blood regeneration, leucopenia, and relative or absolute lymphocytosis In the late stages, an occasional lymphocyte of immature type or even a lymphoblast may be found in the blood, but, in the past, diagnosis was often deferred until autopsy and was sometimes impossible until histological sections were examined Now, sternal puncture permits of an accurate diagnosis of lympha denosis, but the myelogram itself gives no indication that the blood picture is aleukæmic The *marrow* changes are the same wh[illegible] the blood is large or small [illegible] ubt th[illegible] *but* there seems to be much confusion in the literature as to what shall be called monocytic leukæmia, and what *mixed myeloid and mono-cytic* leukæmia In our opinion the term, *monocytic* leukæmia (Schilling type, Plate 5A), should be *limited to those* cases which run an acute or sub acute course (never *chronic*), show a leucocytosis due to an increase in monocytes or their *precursors, and an aplastic*

PLATE 6



A Acute Myeloid Leukæmia
(a) Myeloblast (see p. 4)



B Acute Lymphatic Leukæmia
(a) Lymphoblast (see p. 6)

To face p. 24.

or hypoplastic type of anæmia The so-called mixed (Naegeli, Plate 5B) types are to be regarded as variants of myelogenous leukæmia In these, there occur transitory increases in the number of monocytic elements in the blood and the marrow which are probably atypical myeloblasts, but the general clinical and hæmatological picture, throughout the greater part of the illness, is identical with the myeloid type of leukæmia This is even more evident in tissues examined histologically, here the reticulo-endothelial system shows the changes characteristic of myeloid leukæmia

The marrow films in true monocytic leukæmia are quite characteristic As in all leukæmias there are many cells, monocytes and pro monocytes predominate and form from 70 to 90 per cent of all the nucleated cells The mature monocytes have lobed or rounded nuclei with fine reticular chromatin, and a varying amount of cytoplasm which may or may not contain azurophilic granules The monoblast is similar to, and may be difficult to distinguish from, myeloblasts and lymphoblasts although it commonly shows more variation in size than these cells The promonocyte has a rounded nucleus without nucleoli and may show some azurophilic granules in the cytoplasm The number of mitotic figures is striking (many may even be found in the peripheral blood) and these are chiefly in the pro-monocytes The number of hæmocytoblasts is increased and, indeed, these cells may be closely simulate from the promonocyte if the latter is at all atypical, and in monocytic leukæmia it often is atypical

Erythropoiesis is markedly depressed There is a reduction of all the types of normoblast, but particularly of the earlier and more basophilic forms Megakaryocytes and platelets are scanty From films it is difficult to say whether the granulocytic cells are affected, but quite often the absolute number of neutrophile polymorphs in the peripheral blood is little, if at all, reduced

In the "monocytic" phase of myeloid leukæmia atypical myeloblasts resembling monocytes appear in varying numbers in the blood and marrow As this phase only occurs in the chronic form of myelogenous leukæmia, an erythroblastic hyperplasia of the bone-marrow is to be expected, unless, of course, the malady is in its terminal stages and aplasia of the erythroblastic tissue has occurred

Acute Leukæmia (Plate 6) This disease may be primary or it may be the terminal phase in a chronic case It is usual to distinguish between acute myeloid and acute lymphatic leukæmia, although the differentiation is of purely academic value The

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affects the erythropoietic tissue, there is less difficulty than when the leucoblastic cells are involved In the latter case, it may be difficult to differentiate simple reactions from leukæmia, and, indeed, blood examination is often more important for diagnosis than is marrow puncture It is only if the small focal masses of cells typical of leukæmia are found that a definite conclusion can be reached

Other significant points have to be taken into account Thus, if [illegible]

Even so, the histological arrangement of the cells is different In infections a definite focal distribution is never found Then also, the characters of the more mature cells may help to distinguish a leukæmoid reaction from genuine leukæmia, in the latter they may be abnormal in the sense of being malformed, but they never show the degenerative changes that are so common in infective states

There can be no doubt that there is still need for much investigation of the marrow in the diseases that show a leukæmoid blood picture, and at the present time we are not in a position to say more about them

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CHAPTER III

NEOPLASTIC AND ALLIED CONDITIONS OF THE BONE-MARROW

In so far as they directly affect the myelogram, bone-marrow tumours fall into two groups In the first are *the generalised neoplasms of the hæmopoietic tissue* where sternal puncture may show the marrow to consist almost entirely of tumour cells In the second, the tumours are *localised* and unless a nodule of growth is punctured, the myelogram shows only the effects produced by compression, replacement and irritation, *i e*, leuco erythroblastosis, which may progress to hypoplasia In this group, the tumours may be primary in the bone marrow or metastatic from growths elsewhere in the body, and it *must be borne in mind that it is only when this type* is fairly widespread in the marrow that hæmic erythroblastosis is produced Tumour cells will not be found in marrow films unless a nodule is present at the site of puncture

In our present state of knowledge any attempt to classify bone-marrow tumours must be tentative and somewhat controversial As, in this book, we are concerned chiefly with marrow puncture as a method of clinical and cytological investigation, most of the discussion will be devoted to the most helpful methods of elucidating the myelogram, but the possible relationships of the several conditions must first be considered

Bone marrow is mesenchymal in origin and most of the elements of the reticulo-endothelial system are represented in it, so that a variety of neoplastic conditions may arise as primary "tumours," including the reticuloses, reticulo sarcomata and the myelomata The pathology of the reticuloses has been put on a sound basis by Robb Smith in this country and Gall and Mallory in America Both classifications are cytological, the authors all agreeing that gross anatomical and clinical classifications have no practical value Gall and Mallory classify their cases according to the degree of development of the cells involved, while Robb Smith bases his arrangement on anatomical grounds Thus we have the *follicular reticuloses*, the parent cell of which is the reticulum cell of the germinal follicle of the lymph-nodes, the *sinus reticuloses*, which arise from the living cells of the sinuses of the reticulo-

endothelial system, and the *medullary reticuloses*, which take origin from the free reticulum cells or their descendants, lying in the stroma of the reticulo-endothelial tissue between the sinuses. In the last group there are lymphoid, myeloid and monocytic medullary reticuloses, which we commonly call lymphatic, myeloid and monocytic leukæmia respectively. This group also contains the metabolic reticuloses of Gaucher's disease, Niemann-Pick disease and xanthomatosis. *Fibro-myeloid medullary reticulosis*, or Hodgkin's disease, is yet another component of this group.

The *reticulo-sarcomata*, including lymphosarcoma, should not be regarded as a separate group of diseases but as a more malignant phase of the corresponding cell type reticulosis (Table II).

TABLE II

RELATIONSHIP OF EWING'S TUMOUR AND MYELOMATA TO RETICULO-ENDOTHELIUM OF BONE-MARROW

BONE MARROW CELL	RETICULOSIS	MALIGNANT TYPE
MESENCHYMAL CELL	RETICULUM CELL RETICULOSIS	(EWING'S TUMOUR)
		RETICULUM CELL MYELOMA
HÆMOCYTOBLAST		HÆMOCYTOBLASTIC MYELOMA
ERYTHROBLAST	POLYCYTHÆMIA VERA	ERYTHROBLASTIC MYELOMA
MYELOBLAST	MYELOID LEUKÆMIA	MYELOBLASTIC MYELOMA
PLASMOBLAST	PLASMA CELL LEUKÆMIA	PLASMA CELL MYELOMA

The myelomata form a group of tumours which arise from blood cells or their precursors. The hæmocytoblast, a direct descendant of the primitive mesenchymal cell, is now commonly supposed to be the parent cell of all blood cells and from it arise the blast cells which differentiate to the adult red corpuscle, granulocyte, monocyte, lymphocyte and plasma cell. This line of development is shown in Table III.

Theoretically any of these groups of cells may produce tumours, and hence myelomata, but in practice the term is reserved for those tumours which occur primarily in the bone-marrow. Lymphatic and monocytic tumours occur in the lymphadenoid tissue rather than the marrow and should be considered with the reticuloses. It may be said that plasma cells also are mainly produced in the lymphoid organs, but they also occur in the blood-forming bone-

marrow and glands of cases of acute and chronic myeloid leukæmia The chief significance of the disease is that it presents a typical leukæmic infiltration of the bone marrow and viscera with a leukæmic blood picture and at the same time produces bulky medullary tumours which provide all the essential features of a malignant neoplasm, that is, active *proliferation atypical cells*, distant metastases, some of which are clearly of embolic origin, and destructive local infiltration In fact, the disease is a mixture of leukæmia and malignant myeloma

In another group of cases with radiologically typical myelomata a leukæmic or subleukæmic blood picture is present In fact, Piney and Riach have described four grades of *plasma cell myeloma* depending on the type and extent of bone involvement and the condition of the blood These are —

(1) Much bone destruction and no blood changes
(2) Moderate bone destruction and a few plasma cells circulating in the blood stream
(3) Bone destruction less with a diffuse marrow infiltration and a larger number of circulating plasma cells
(4) Diffuse bone-marrow infiltration without erosion and a frankly leukæmic picture in the blood and viscera This is plasma cell leukæmia

A third group of cases which appear intermediate between the two conditions are those whose peripheral blood picture and sternal marrow are typical of leukæmia but there is no enlargement of the spleen or liver In these, X-ray of the long bones will often reveal several small areas of rarefaction with loss of bone pattern, suggesting a mild degree of erosion by local deposits Fig II is an X-ray of the hand of one such case The patient was a girl aged about eight years old who was originally seen complaining of pain in the hands and feet Complete radiological examination of the skeleton showed only the lesions in the figure and similar areas of rarefaction in the 5th metatarsal bones The diagnosis of leukæmia was confirmed by blood count and marrow puncture The girl died at home within a year and no post mortem examination was possible

These intermediate types of case form a definite link between leukæmia and multiple myeloma and are probably more common than is generally realised Many conditions labelled simply leukæmia are atypical when closely studied and the deviations from the classical picture are often towards the myelomatous syndrome

FIG II



EROSION OF BONE IN LEUKEMIA

Ewing's tumour, or endothelioma of bone, must also be considered in any discussion of myelomata In this disease, tumours growing within the marrow cavities of the bones cause irregular absorption of the shaft and widening of the marrow cavity In the later stages the bone may be completely destroyed The regional lymph nodes *are often involved and pulmonary metastases occur in nearly all* cases The clinical course of the disease is more rapid than with myeloma and the tumours are more invasive

Ewing himself thinks the tumour arises from the endothelium of the blood vessels of the bone marrow, but other authors, notably Oberling, claim to have observed differentiation of the tumour cells into plasma cells, myelocytes and even primitive red cells If this *is so the tumour may well be an hæmocytoblastoma or myeloma of* the most primitive blood cell, especially as these tumours commonly occur in the ends of long bones in children and infants—that is during the age period when these bones contain hæmopoietic marrow

As the hæmocytoblast is a direct descendant of the reticulum cell and mesenchymal cell, which also may form the endothelial lining of blood vessels, a reticulum cell myeloma in the bone marrow may well show angioblastic tendencies in some cases, thus accounting for the features stressed by Ewing As also the primitive mesenchyme is situated perivascularly, they may be included in the "perivascular endothelium,' which Ewing cites as the possible origin of these tumours In fact, the implications of a multipotent primitive cell in the neoplastic process is the only way to account for the varying ways in which these tumours tend to differentiate

Those tumours of bone-marrow which are included in the group known as Ewing's tumour may therefore be classed as myelomata of the more primitive cells of the reticulo endothelial system in the bone marrow and any of them may tend to show differentiation along the lines of the more adult bone marrow cells The greater clinical malignancy of these tumours is accounted for by the more primitive nature of the cells involved This group may be regarded as the sarcomatous lesion of these primitive reticulo-endothelial cells as opposed to the systematised overgrowth known as reticulum cell reticulosis, which *in the bone-marrow is represented by the multiple endothelioma of bone first described by Marckwald* The cells are the same clear polyhedral cells as seen in Ewing's tumour, and although death occurs in two years the tumour does not show the usual features of malignancy like Ewing's tumour

The patients die of anæmia and cachexia with severe pyrexia—a type of illness which compares closely with the condition seen in animals when the R E S is disorganised by blockage with dye, etc

In the bone-marrow, therefore, we can demonstrate a *relationship* of the different tumours in the same way as can be demonstrated in the rest of the reticulo-endothelial system This *relationship is shown in Table V Table IV shows a more complete* classification, including the other reticuloses and their sarcomatous manifestations

TABLE V

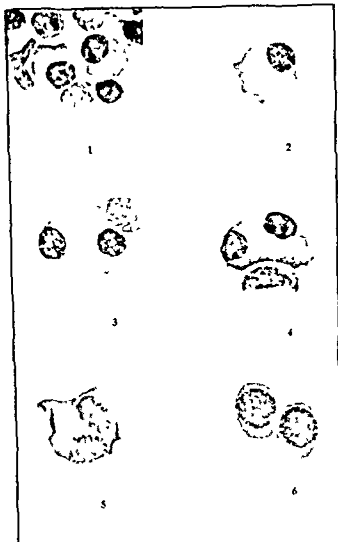
HYPERPLASTIC AND SARCOMATOUS RETICULOSES

MEDULLARY RETICULUM	RETICULUM CELL RETICULOSIS *HISTIOCYTIC RETICULOSIS*	RETICULO-SARCOMATA (EWING'S TUMOUR IN BONE MARROW)
	LYMPHOID LEUKÆMIC NON LEUKÆMIC	LYMPHOSARCOMA
	MYELOID LEUKÆMIC NON LEUKÆMIC	MYELOBLASTIC MYELOMA
	PLASMA CELL LEUKÆMIA	PLASMA CELL MYELOMA
	ERYTHROID POLYCYTHÆMIA VERA	ERYTHROBLASTIC MYELOMA
	LYMPHADENOMA	*HODGKIN'S SARCOMA*

Myeloma. There are four generally recognised types of myeloma —

(i) plasma cell,
(ii) myeloblastic, derived from the primitive myeloid cells,
(iii) erythroblastic, derived from the nucleated red cells;
(iv) lymphoblastic, derived from the lymphoid cell series, *and* which is probably secondary to lympho sarcoma elsewhere

PLATE 7



PLASMA CELLS FROM CASE OF PLASMA-CELL MYELOMA

1 Plasmoblasts
2 Mitotic division of plasmoblasts
3 Binucleate plasma cell
4 Binucleate plasma cell
5 Normal plasma cells
6 Plasma cell with cytoplasmic vacuola

[To face]

The majority of myelomata are composed of elements resembling plasma cells indeed, there is little reason for doubting that they are really plasma cells But, according to one's opinion, these tumours would equally properly be designated as pseudo plasmacytomata

We have already seen that the nature of plasma cells is still uncertain (p 6), though that need not affect our descriptive knowledge of tumours composed of them, but it must be emphasised that it is often possible to observe what appears to be a continuous series of stages between the cells of the reticulo-endothelium and typical plasma cells But, of course, these elements, as seen in [illegible]

which is vacuolated round the nucleus (*heller Hof*) In films, there is much more variation in size, some of the cells being no larger than small lymphocytes, whilst others are as large as monocytes, and may (in marrow but not in blood) contain more than one nucleus The basi chromatin is much more variable in its distribution than it is in the plasma cells of the tissues it may be "cart-wheel," but it is often quite irregular, similarly, it may be symmetrically or excentrically placed in the nucleus The cytoplasm is basophilic, but less intensely so than in the tissues it may be vacuolated but not necessarily in the vicinity of the nucleus, and occasionally it is seen to contain azurophilic granules such as rarely occur in tissue plasma cells Mitotic figures are often observable Azurophilic protein crystals may be found in a few "myeloma cells" (Steinmann)

Marrow films, thus, present a very varied appearance, which is extremely suggestive of neoplasia, but which does not always immediately suggest that the predominant element is the plasma cell But, even so, a little experience allows a diagnosis of myeloma to be made with much greater certainty than is possible even with X-rays (Rosenthal and Vogel) Of course, the film does not consist entirely of plasmacytoid elements it is very rare for all the ordinary marrow cells to be replaced As a rule about 50 per cent of the cells are of plasma cell type But, if the needle has penetrated a nodule of "tumour," it may be found that almost every cell is of this kind But, from the point of view of diagnosis, these differences are of no special significance

In the *myeloblastoma*, the cells are, on the whole, larger and may contain few or many neutrophilic granules in the cytoplasm Many

polymorphonuclears and myelocytes are present, and both may be found in the peripheral blood

In the *erythroblastoma*, the normoblasts are usually well hæmoglobinised, but there may be a large percentage with basophilic cytoplasm The cells are small, and the nuclei pyknotic It is still uncertain whether erythroblastic tumours, in which there are no hæmoglobinised elements, really exist

Snapper and his co-workers have shown that treatment of multiple myeloma with stilbamidine often results in the development of peculiar changes in the plasma cells in the marrow These take the form of basophilic granules in the cytoplasm Similar changes occur after treatment with antimony as a specific result of the effect on ribose nucleic acid in which the cytoplasm of myeloma cells is rich It would appear, therefore, that the nucleoprotein of myeloma cells differs from that of other cells which do not react in this way with antimony or stilbamidine

Chloroma As a rule, symptoms and signs suggestive of acute or sub-acute leukæmia precede the development of chloromatous tumours , but, occasionally, there are no changes in the blood, except perhaps slight hypochromic anæmia, even at a time when quite large masses are present in the orbits Then, examination of the marrow is likely to be very helpful, especially as in this disease, mitotic activity is remarkably intense The tumour cells are scattered throughout the marrow, some being isolated, others in groups of six or nine , and the adjacent myeloid tissue is distinctly hyperplastic Later, when the tumour has spread, the greater part of the marrow may be replaced by abnormal cells , and it is then difficult to discover any remnants of normal tissue

The characteristic cells are of the same type in every case of chloroma They are large, with a bulky nucleus, which contains distinct nucleoli The basi-chromatin has an almost reticular distribution, and the cytoplasm, which is moderately basophilic, contains no granules Cells identical with those in the marrow are only rarely found in the circulation, where the predominant cells are usually myeloblasts or extremely immature premyelocytes It is this latter fact that suggests that all cases of chloroma are of myeloid nature, although some writers still contend that lymphatic examples do occur It is, however, more probable that chloroma represents a state intermediate between true acute leukæmia and a neoplastic condition

Reticulo-Endotheliosis. The *follicular reticuloses* produce no

change in the peripheral blood picture, and it is only when secondary nodules develop in the bone marrow that we get a picture of leucoblastic and erythroblastic hyperplasia, which may eventually develop into aplasia of the marrow if the invasion by tumour becomes more or less complete

The sinus reticuloses also show no abnormality in the peripheral blood, apart from moderate anæmia and slight thrombocytopenia, but they do cause marked changes in the bone-marrow For this reason, Dameshek, who has reviewed most of the recorded cases, refers to them as *aleukæmic sinus reticuloses* The bone-marrow presents a varied picture Characteristically, there are small collections of large cells, 20 to 30 microns in diameter, each with a rounded or indented nucleus, which occupies more than half of the [illegible]

[illegible]

of the cells show azurophilic granules in the " Hof " of the nucleus Mitotic figures are frequently seen The myeloid and erythroid elements are decreased, and megakaryocytes are absent. This probably represents a hypoplastic or destructive condition of the bone-marrow, and it is interesting to note that in none of the recorded cases has there been an initial hyperplasia It seems a reasonable inference that the tumour cells are not merely replacing the normal marrow elements, but that they arise from a primitive cell, which is also the precursor of the blood cells consequently, on account of this perversion of function, the hæmopoietic tissue is unable to react in the ordinary way.

Of the *medullary reticuloses*, the leukæmias are discussed elsewhere (p 15)

Gaucher's disease usually affects the spleen more intensely than the bones, although skeletal affection may occasionally predominate But, even in the clinically splenomegalic cases, some involvement of the bone-marrow is common How usual it is was not realised until sternal puncture became a fairly common procedure. This frequency of skeletal involvement is an important matter, because it has been *suggested that splenectomy, in such a metabolic disease as this,* would accelerate the deposition of the Gaucher substance in the bones, so that the last state might well be worse than the first. Logan has shown that the fear is unfounded and that there is, in fact, improvement in almost every case after removal of the spleen

The Gaucher cells in the marrow are large (50 microns or more).

The nuclei present no characteristic arrangement of the chromatin, but are always relatively small and dark, several may be found in one cell The cytoplasm is bulky, with sharply defined outlines Some of the cells are polygonal, others elongated, but few transitional forms are seen In paraffin sections, the cytoplasm has a foamy appearance, due *to solution of the contained lipoid material*, and a faint, diffuse, iron reaction can often be obtained There seem to be three fairly distinct stages in the development of these elements Thus, the least differentiated cells have granular cyto plasm, in which lies a more or less reticular nucleus Then, there are cells with similar cytoplasm, but dark and almost structureless nuclei, and lastly, the fully developed Gaucher cell, with vacuolated cytoplasm and small dense nucleus It is only in the last type that it is possible to detect the presence of fine fibrils, which are best shown by silver-impregnation methods

The myeloid tissue itself is usually hyperplastic, in contrast to the peripheral leucopenia, and there is a slight increase of monocytes (up to 10 per cent) in the myelogram This is not sufficient by itself to arouse a suspicion of Gaucher's disease, but it does give *support to the view that monocytes may arise from the same primitive* reticulum cell

Niemann Pick disease shows a similar invasion of the marrow with " tumour " cells, and hyperplasia *of the myeloid tissue In contrast* to Gaucher's disease, however, there is usually a peripheral leucocytosis The characteristic cells are large (20 to 80 microns) and contain a reticular nucleus The cytoplasm is *filled* with droplets that give rise to a " foamy " appearance very different from the fibrillary appearance of the typical Gaucher cells As the disease is always fatal by the age of two years, marrow biopsy must be done by tibial trephine, or smears must be made from spleen puncture

Hodgkin's disease affects lymphatic glands so much more obviously than it does the marrow, that relatively little attention has been paid to the latter tissue And, although we probably never obtain assistance in diagnosis by examining the bone marrow, there are points of interest and importance in connection with it

The cases fall *into* four groups, viz, those with normal marrow, [illegible], those with lymphadeno-

In the third group it is not, as a rule, possible to be certain that

dyshæmopoietic In the following chapters the main changes from [illegible]

[illegible] heading

Hæmorrhagic Anæmia Anæmia following hæmorrhage may be acute or chronic, depending on the amount, frequency, and duration of the bleeding Acute anæmia is most commonly seen following trauma, bleeding from a peptic ulcer, or childbirth Providing that treatment is adequate, the blood loss is replaced in about thirty days and this replacement is brought about by an increase in the normal activity of the bone marrow

The chronic type, usually induced by long continued, frequent and relatively small bleedings, such as occur in menorrhagia and some types of peptic ulcer, produces characteristic changes in the myelogram A study of these two types of marrow change helps us to interpret the findings in hæmorrhagic anæmias due to abnormalities of the blood or blood vessels, of which thrombocytopenic purpura and hæmophilia are examples The special findings peculiar to these anæmias will be discussed later Certainly the myelogram in traumatic anæmias does not help in diagnosis, either of the nature of the anæmia (which is usually obvious on clinical grounds) or of the site of the loss of blood

Acute hæmorrhagic anæmia The myelogram here shows increased normal activity Both the erythroblastic and myeloid elements are affected, the former more than the latter The hyperplasia of the myeloid tissue is reflected in the peripheral leucocytosis

The normoblasts are increased in number and show a tendency to become hæmoglobinised at an earlier stage, *i e*, the usual basophilic normoblast becomes polychromatic, and the polychromatic one becomes eosinophilic The number of reticulocytes present is markedly increased Megakaryocytes are more frequent and may be seen in moderately large numbers in the thick end of the film In the percentage count the polymorphs are decreased, but this decrease is only apparent because the total number of cells is increased Mitoses are more numerous, indicating general hyperplasia The mitoses, both of red and white cells, takes place at the normal level of cell development The myelogram is as follows —

Neutrophiles	
Myelocytes	30-35 per cent
Metamyelocytes . .	10-15 „ „

Eosinophiles	
Myelocytes	1– 2 per cent.
Polymorphs	1– 2 " "
Basophiles.	
Myelocytes	3– 6 " "
Polymorphs	1– 2 " "
Premyelocytes	1– 2 " "
Hæmocytoblasts (including myeloblasts)	2– 4 " "
Lymphocytes	6–12 " "
Pro-erythroblasts	1– 2 " "
Normoblasts	25–30 " "
Megakaryocytes	frequent

Chronic Post hæmorrhagic Anæmia When the anæmia is but moderate the bone marrow activity is only slightly increased In more severe cases there may be a great increase in the amount of red marrow This hyperplasia may eventually be succeeded by exhaustion, and the marrow then becomes hypoplastic or aplastic In a fully reacting case, i.e, before marrow exhaustion supervenes, [illegible] numerous mitotic figures of normal type The myelocytes are little, if at all, reduced in number, and the total number of cells is somewhat increased Reticulocytes are fairly numerous though *not* as abundant [illegible] that iron is needed for complete maturation of the cells, and not as a stimulus to erythropoiesis itself

Neutrophiles	
Myelocytes	20 –30 per cent
Metamyelocytes	5 – 8 " "
Polymorphs	8 –12 " "
Eosinophiles	
Myelocytes	1 – 2 " "
Polymorphs	1 – 2 " "
Basophiles	
Myelocytes	½– 3 " "
Polymorphs	½– 3 " "

Premyelocytes	1 – 3 per cent
Myeloblasts	0 – 2 " "
Pro-erythroblasts	3 – 5 " "
Normoblasts	40 –50 " "
Megakaryocytes	moderate

Hæmorrhagic Anæmias The specific marrow changes in *essential thrombocytopenic purpura* will be described in the chapter on aplasia of the bone-marrow

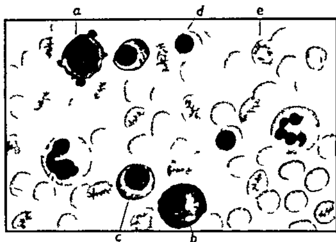
In true *hæmophilia*, apart from the changes described above, the marrow films show a marked increase in the number of megakaryoblasts and megakaryocytes, but the number of platelets is within normal limits Custer and Krumbhaar have described the appearance of the bone-marrow in three fatal cases Limarzi, Poucher and Birch have shown that sternal puncture can be safely performed on hæmophilics and they confirm the findings described

Hæmolytic Anæmia (Plate 8A) In all forms of hæmolytic anæmia the activity of the marrow is at its maximum There is great and continuous normoblastosis which is presumably induced by loss of corpuscles, due to destruction in the body but whether any erythropoietic stimulus is liberated by the destroyed cells is still uncertain, although probable

The clinical state is not clearly, if at all, reflected in the marrow, which is in a condition of extreme activity throughout the course of the malady About three quarters of the cells in the marrow are nucleated red-cells in various stages of development, some are mature, but the majority are basophilic and possess large cartwheel nuclei Another striking feature is the presence of considerable numbers of cells as immature as the pro erythroblast these, as previously described, differ from megaloblasts but are at least as large, if not larger In some cases a true megaloblastic reaction may develop as described by Fairley in cases of Bartonella fever The large number of reticulocytes is another characteristic feature

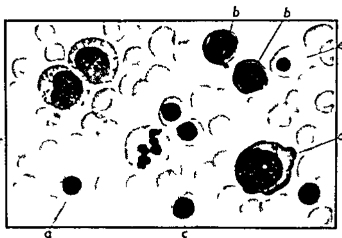
The myelogram is of the type shown below :—

Neutrophiles	
Myelocytes	10–17 per cent
Metamyelocytes	2– 5 " "
Polymorphs	2– 5 " "
Eosinophiles	
Myelocytes	0– 2 " "
Polymorphs	2– 5 "



A HÆMOLYTIC ANÆMIA

(*a*) Pro-erythroblast
( ) Basophilic normoblast
(*b*) Plasma cell
(*d*) Normoblast
(*e*) Reticulocyte



B SPRUE

(*a*) Promegaloblast
(*d*) Normoblast
(*b*) Early normoblast
( ) Eosinophilic megaloblast
( ) Basophilic normoblast
(*f*) Megalocyte

Lymphocytes	1– 2 per cent
Monocytes	1– 2 „ „
Premyelocytes	5–10 „ „
Hæmohistioblasts	1– 3 „ „
Hæmocytoblasts	1– 4 „ „
Pro-erythroblasts	25–34 „ „
Normoblasts	20–30 „ „
Megaloblasts	0– 2 „ „
Megakaryocytes	not increased

Reticulocytes numerous May be as high as 80 per cent of the non-nucleated elements of the films

In many of the hæmolytic anæmias the anæmia may be macrocytic in type In all the groups one or more such cases are described in the literature Apart from the true megaloblastic reaction in Bartonella fever, however, the characteristic feature of these marrow films is the high percentage of pro-erythroblasts Limarzi has shown that an intense normoblastosis occurs in the marrow in the rare hæmolytic anæmia following sulphonamide poisoning and is accompanied by a macrocytic anæmia The macrocytosis is, therefore, probably due to rapid maturation of the pro-erythroblasts and basophil normoblasts, the nucleus being extruded rather early in the normoblastic cycle That it is not due to megaloblastosis is borne out by the fact that liver preparations do not cause improvement

In the *infective group of hæmolytic anæmias*, the myelogram does not show such a high proportion of pro-erythroblasts, and the regeneration of red cells is not as a rule so intense The hæmolysis is probably due to the infecting organism causing a "toxæmia" of the red cells which render them more liable to the normal process of destruction This toxic action is also exerted on the bone marrow, which cannot then respond so rapidly Where the infection is prolonged and the toxæmia extreme, the marrow fails to respond at all and becomes aplastic (*erythronoclasia*) We have recently seen a case of this type associated with tuberculosis, and sections of the bone-marrow removed at autopsy showed a few minute tubercles

Gas gangrene infection (*Cl Welchii*), and sometimes an anaerobic hæmolytic streptococcus, produce a hæmolytic anæmia in a different way They produce a hæmolysin which acts directly on the red cells In these cases, until the toxæmia becomes severe, the marrow may be expected to react more exuberantly

Malaria produces a chronic hæmolytic anæmia, the hæmolysis being caused by direct action of the *parasite on the red corpuscles* Even in malignant tertian infection, where the hæmolysis may be so intense as to produce blackwater fever, and early death, the marrow shows a well marked erythroblastic hyperplasia with large, intensely basophilic, normoblastic cells (see Chapter VIII)

Some hæmolytic anæmias are due to poisons lead, potassium chlorate, benzedrine, phenylhydrazine, phosphorus, T N T, dinitrobenzene, etc The reaction of the bone marrow to all these compounds is as that described for hæmolytic anæmias in general Aplasia develops rarely, if ever, because the patient dies of some other toxic effect of the poison before the aplastic stage is reached, although a small number of workers with trinitrotoluene *have died of aplastic anæmia The sulphonamide* group of drugs, however, may produce a hæmolytic anæmia, and, if still more is given, the marrow may become aplastic

In lead poisoning, of course, the characteristic basophilic stippling of the red cells will be seen in marrow films, and Henning and Keilhack have reported ten cases where the stippling was found in the red cells of the marrow when absent in the peripheral blood.

The hæmolysis produced by incompatible transfusions and paroxysmal hæmoglobinuria causes no peculiar abnormalities in the bone-marrow

In *acholuric jaundice*, in both the congenital and acquired type, no new information about the pathology of the disease has been acquired by studying bone-marrow films The spherocytosis can be seen as easily as in the peripheral blood A constantly high reticulocyte count may be found in the marrow even when the red cell count is nearly normal The reticulocyte count may be normal during a remission of the disease and the spherocytosis may temporarily diminish Spherocytosis is not so marked in the acquired form of the disease

It has always been assumed that the crises that occur *in congenital* hæmolytic jaundice are due to sudden great increase of destruction of the red cells This view has recently been challenged by Owren, who alleges that the rapid increase of anæmia is probably due to a temporary hypoplasia of the bone-marrow And he has shown *that* the marrow changes at the onset of such a crises *strongly support* this view

Just before the crisis, the marrow shows the usual intense erythropoiesis of the disease, but within two or three days there

are practically no erythroblasts remaining in the marrow, then, as the crisis dies down, there is regeneration of pro-erythroblasts which mature into macroblasts, until, at the end of the crisis, the marrow shows an enormous increase of normoblasts, while at the same time, the number of reticulocytes in the circulating blood rises sharply Owren's views, although not yet confirmed, would explain the low percentage or absence of reticulocytes that has long been known to occur during crises

In *sickle cell anæmia*, the latent phase is much more common than the active one In the latent form, sickling of the cells is not found in the peripheral blood but has been found in the bone-marrow of a case in this phase coming to autopsy The presence of sickle cells is not diagnostic of anæmia, as the sickle cell trait has been found in 5 7 per cent of all negroes, whether or not they are in good health The phenomenon of sickling cannot ordinarily be seen in stained films A fresh preparation sealed under a cover slip should be examined a few hours after it is made The myelogram does not differ from that of any other form of hæmolytic anæmia except, of course, in the shape of the red corpuscles Perhaps, however, the number of monocytes containing red corpuscles or pigment is rather greater, and Wintrobe has recorded the presence of long bands of erythrocyte cytoplasm (about 2 microns thick) lying in marrow films

In *Lederer's anæmia* the marrow picture may be complicated by the presence of a leukæmoid reaction This is usually myeloid in type, but a case has been described with the appearances of lymphatic leukæmia in the peripheral blood, including lymphoblasts

In *icterus gravis* the marrow changes are the same as in any hæmolytic anæmia Marrow puncture is not of much value because the peripheral blood reflects the condition of the marrow in showing a variety of normoblastic cells which leave no doubt as to the diagnosis Besides, sternal puncture and tibial trephine are not suitable procedures to be employed on new-born babies

Toxic Anæmias. This group contains the hypoplastic and aplastic anæmias It is a rather heterogeneous group of maladies, which may be primary or secondary If of the latter type, the cause may be poisoning of various kinds, infection or toxæmia But, whatever the cause, the malady is a grave and dangerous one, and, in all, the marrow picture is similar, no indication of the cause can be inferred from the myelogram The conditions are more described later with other aplastic conditions of the bone marrow

Dyshæmopoietic Anæmias. The hæmopoietic bone-marrow is a complicated and highly specialised structure which has to supply the formed elements of the blood continuously and rapidly In order that it may do this efficiently, an adequate and properly balanced supply of nutriment is necessary This has long been recognised, but our knowledge of the food requirements of the blood-forming tissue has, *until the end of the first* quarter of this century, been confined to one constituent, iron Now we know that the hæmopoietic principle, copper and other metals, thyroxin, and vitamin C all play their part Lack of, or failure to utilise, any of these factors may result in anæmia As a result of malnutrition of the bone marrow, two main groups of anæmia are produced (1) hypochromic and microcytic, (2) hyperchromic and megalocytic *The first group* arises with *deficiency* of iron, internal secretion of thyroid, and vitamin C The second arises with deficiency of the hæmopoietic principle

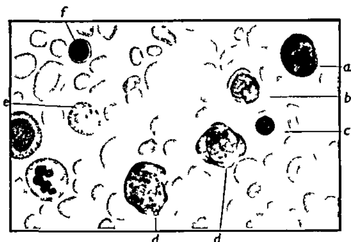
Deficiency of hæmopoietic principle —

Pernicious anæmia (Plates 9, 10 and 11) is, of course, the best known anæmia of this group An exactly similar condition of the blood and marrow may obtain in other anæmias of the same type, whether the cause be carcinoma of the stomach, complete gastrectomy or other conditions giving rise to deficiency of the hæmopoietic principle

The myelogram in pernicious anæmia is striking and characteristic The constant feature is the presence of megaloblasts, but it is rather on the large number of these cells than on their simple presence that diagnosis is to be based As mentioned earlier, some writers aver that a few of these cells can be *found, even in health, it is, therefore,* an increase that is significant But there is another point even if the megaloblast be not regarded as peculiar to pernicious anæmia, there is no doubt that the marrow contains a larger number and far more young forms in this disease than are found in any other It must not, however, be supposed that the whole of the erythropoietic process in this disease is megaloblastic in type, there is great normoblastic activity also *in other words, there is a mixed erythroblastic* reaction of an intensity never seen in other *maladies*

Little is known of the changes in very early cases, but Segerdahl states that the characteristic blood picture can be found before there are any recognisable changes in the marrow But in definite, and untreated cases, the myelogram is very striking, inasmuch as there are two changes intense megaloblastosis which of course, is

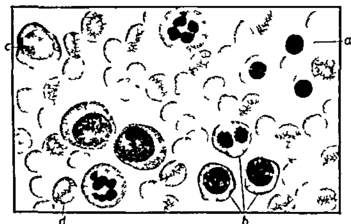
PLATE 9



A Pernicious Anæmia (Untreated)

(a) Early megaloblast
(b) Late megaloblast
( ) Pyknot c megaloblast
(d) Intermed ate megaloblast
(e) St ppled megalocyte
(f) Lymphocyte

Note —Megalocytos s po k locytos s and an socytos s



B Pernicious Anæmia (Early Stage of Treatment)

(a) Intermed ate normoblast
(b) Early normoblasts
( ) Intermed ate megaloblast
(d) Ret culocyte

Note —Megalocytos s less marked than in A

[*To face p* 44

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pathognomonic, and a leucoblastic reaction of a non-specific kind

It is surprising that the latter change should occur, because at this stage there is leucopenia with relative lymphocytosis in the blood This may be due to some inhibition of emigration and also of proper maturation of the granular leucocytes Certainly it is common to be able to detect abnormalities of both nuclei and cytoplasm in the neutrophiles, especially in the metamyelocyte stage (p 6)

Fallon regards the changes in the metamyelocytes as diagnostic of pernicious anæmia, and Dameshek and Valentine say that the percentage of these cells increases with the severity of the disease The nature of the changes is uncertain, but on the available evidence we would agree with Jones that the abnormal cells are the result of abnormal development of the myelocytes associated with the deficiency of hæmopoietin Tempka and Braun, who originally drew attention to the altered metamyelocytes, regarded the changes in them as degenerative, and Nordensen concurs with this opinion The metamyelocytes are increased in size up to 30μ and show large U shaped nuclei with a coarse chromatin network Cytoplasmic granules are present but only stain well when a *p*H 6 8 buffer is used for diluting the neat stain (Foy and Kondi),

Further, there is a considerable excess of hæmocytoblasts, which, of course, adds to the appearance of leucoblastic activity In some long-standing, untreated, cases, there may be some depression of granulopoiesis

There is much difference of opinion as regards the megakaryocytes in this malady, and at the present time it is best to assume that they may or may not show changes The presence of many naked megakaryocytic nuclei has been commented on (Tempka and Braun), and Jones (1936) found some intensely basophil megakaryocytes with no granules in the cytoplasm and marked pleomorphism of the nucleus Other workers have merely noted a diminution in the number of megakaryocytes, while Nordensen holds that they are normal in appearance and number

Japa claims that the essential abnormality in pernicious anæmia is a slowing down of the rate of mitosis with increase in the length of the resting phase and consequent increase in size of the affected cells, all three cell types, erythroblastic, leukoblastic and megakaryoblastic are affected This change is said to be due to an alteration in nuclear metabolism

The megaloblastosis is, naturally, the most significant change,

and it is by progressive increase that we can recognise deterioration by diminution, that we see that improvement is occuring and by disappearance that complete relief is established The cells are easily distinguished from normoblasts, but too much attention should not be paid to their size, which is very variable The nuclear characters (p 9) are the differential feature These cells are found lying in groups, often surrounded by a clear area, devoid of cells, and they seem to develop more or less independently of all other marrow elements Promegaloblasts are also present, and their number is some guide to prognosis It is interesting to find that the number of these cells is closely related to that of the hæmocytoblasts, as the one decreases, so do the others And it is reasonable to assume that they are closely related, and indeed, probably of the same series

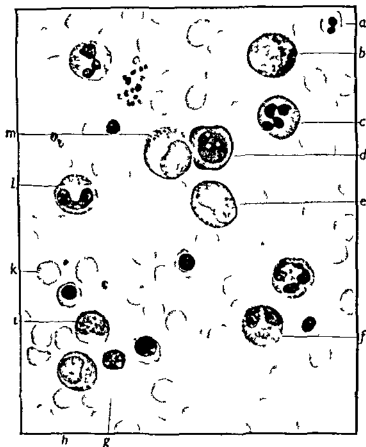
In an untreated case of moderate or great severity, the striking feature of the marrow film is that there is a general immaturity, and *the marrow appears to be both hyperplastic and metaplastic*, the latter appearance being due to the presence of so many hæmocytoblasts Indeed, so many of the marrow cells are immature, that, with the ordinary hæmatological stains, the films have a strikingly blue colour, which, by itself, is very suggestive of pernicious anæmia In myeloblastic leukæmia, the films are far less blue, because the myeloblast has a cytoplasm somewhat less basophilic than the hæmocytoblast and the promegaloblast

Of course, the other signs of pernicious anæmia to which we attach so much importance in the blood, can also be detected in the marrow megalocytosis, anisocytosis, Jolly bodies, hyperchromia and polychromasia are always seen *If the marrow fluid is stained* with cresyl blue (see Appendix) before the films are made, there is a *distinct though moderate increase* in the number of reticulocytes

Mitotic figures are often numerous, and so far as it is possible to be sure, they occur mainly, or perhaps entirely, in the megaloblasts But the number of atypical mitoses is always great some are pyknotic, some multipolar, and others even more grossly irregular This is pathognomonic of pernicious anæmia, and is never seen in the normoblastic mitoses that may be numerous in post hæmorrhagic anæmia during the phase of regeneration

In the phases of remission, induced by liver treatment, a series of significant changes *can be detected These start* about twenty four hours after intramuscular injection of a potent liver extract, and *the first change* to be detected is increase in the number of reticulocytes After 4 c cm of Neo-Hepatex, we have seen the reticulocytes

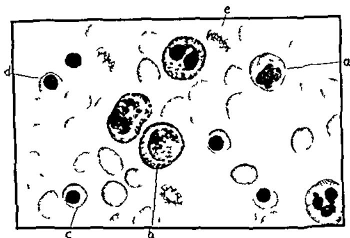
PLATE I



NORMAL MARROW

- (a) Normoblast
- (b) Basophile myelocyte
- (c) Polymorph
- (d) Myeloblast
- (e) Myelocyte
- (f) Eosinophile
- (g) Basophile normoblasts
- (h) Pro-erythroblast
- (i) Early normoblast
- (k) Polychromatic corpuscle
- (l) Metamyelocyte
- (m) Premyelocyte

PLATE 10



A PERNICIOUS ANÆMIA (LATE STAGE IN TREATMENT)

(a) Late megaloblast
(b) Myelocyte
(c) Normoblast
(d) Polychromatic normoblast
(e) Reticulocyte



B IDIOPATHIC HYPOCHROMIC ANÆMIA

Note —Numerous basophilic normoblasts and hypochromic microcytosis

rise from 8 per cent of the total non-nucleated elements to 39 per cent twenty seven hours after the injection Within two days there are signs of a change in type of the erythropoiesis the intense megaloblastosis is beginning to die down and to be replaced by normoblastosis The percentage of the latter cells may rise from twenty-two to thirty-seven in fifty hours Even four to five days after treatment has commenced, the marrow picture is still characteristic of pernicious anæmia About half of the normoblasts are large and have an intensely basophilic cytoplasm these are of the early and basophil type of normoblast The remaining half are composed of equal numbers of polychromatic and eosinophil normoblasts of normal size Mitosis is still intense, but is now confined almost entirely to the large basophil early normoblasts Promegaloblasts and basophil megaloblasts are still present in moderate numbers and are easily recognised by their nuclear characters Very few of them show mitosis Polychromatic and eosinophil megaloblasts are conspicuous only by their absence A few megakaryocytes can now be found in the thick end of the film The activity of the myeloid tissue does not show much change, but in some cases there may be a marked increase in the numbers of eosinophile myelocytes and polymorphs This may be reflected in the peripheral blood picture

After seven to ten days the marrow picture becomes normal The changes in the myelogram, with both anahæmin and folic acid, are shown in Table VI

It is not at all clear by what means the change in erythropoiesis comes about, but it is probable that it is due to a stimulus to normal maturation of young normoblasts, rather than to fresh formation of cells The intense karyokinesis seen in these cells supports this supposition, and the failure of hæmoglobinisation of the megaloblastic cells shows that they are no longer required as oxygen carriers

In the late stages of treatment and in the stage of remission during continued treatment, the marrow film is rather uninteresting (Plate 10A) The megaloblastosis has disappeared, although a very occasional megaloblast may be found before the anæmia has completely disappeared The myeloid elements form a normal proportion of the film Normoblasts are present in normal proportions, but there is a tendency for them to become hæmoglobinised earlier, and for the nucleus to be discarded while the cell is still large This phenomenon probably accounts for the macrocytosis, which still persists in some treated cases of this disease We have already seen

TABLE VI

BONE MARROW CHANGES DURING TREATMENT OF PERNICIOUS ANÆMIA

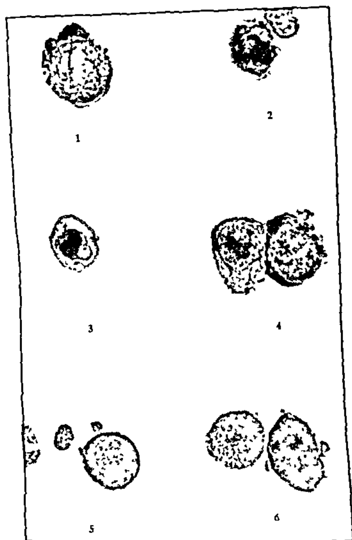
Therapy	Folic Acid 20 mgm. daily for 2 days 10 mgm. daily for 6 days				Anahæmin 1 dose 10 c c				
Time	0	1 day	0 days	16 days	0	6 hours	12 hours	1 day	7 days
Hæmohistioblast	0 6	1 0	0	0 4	0	0	0	0	0
Hæmocytoblast	2 2	1 8	0 4	0 4	0	0 5	0	0 5	0
Myeloblast	2 4	1 2	0 4	0 6	0 5	0 5	0 5	0 5	0 5
Premyelocyte	3 4	0 6	0 8	0 8	0 5	0 5	0 5	0	0 5
Myelocyte	6 6	6 0	5 8	4 0	5 0	8 0	9 5	7 5	14 0
Metamyelocyte									
Polymorphs									
Eosinophil myelocytes									
Eosinophils									
Basophils									
Giant metamyelocytes	12 0	3 4	0	0 2	not recorded separately				
Pro erythroblasts	1 6	3 6	0	1 0	1 5	0 5	0 5	1 0	0·5
Basophil megaloblast	3 0	8 2	1 2	0	8 0	6 5	7 5	8 0	0 5
Polychrome	3 6	6 0	0	0	12 0	15 5	9 0	10·5	1·0
Eosinophil	3 4	4 4	0	0 4	7 0	15 5	3 5	2 5	0 5
Basophil normoblast	0	3 8	1 6	1 8	1 0	1 0	9 5	25 0	3 5
Polychrome	0	0 6	15 0	5 8	3 0	1 0	4 5	10 0	6 0
Eosinophil	0 2	2 2	5 8	1 6	21 0	9 0	6 5	9 0	17 5
Lymphocytes	28 6	22 4	20 4	25 6	8 0	10 0	6 5	4 5	10·5
Plasma cell	0 4	0 2	0	0	0	0 5	0 5	—	0 5
Monocytes	0	0	2 2	0 2	0	0	0	0	0
White red ratio	1 42	0 53	0 7	0 98	0 69	0 80	1 15	0 42	1 2
Mitosis red *cell*	0 2	2 4	0 6	0	0 1	0 3	0 4	1 5	0 2
white cell	?	?	?	?	0	0 2	0 2	0 1	0 1

The figures for folic acid treatment have been reported by Harrison and White
The anahæmin figures are our own

that this mechanism may account for some of the macrocytic hæmolytic anæmias, and it may be that there is still some further factor concerned with normoblastic development with which we are not yet acquainted Reticulocytes in fully treated cases number about 3 per cent in bone marrow films

The practical purpose of sternal puncture in pernicious anæmia is not usually that of early diagnosis, however, in cases which have become hypoplastic, and have an atypical blood picture, marrow puncture is diagnostic As has been said, in ordinary cases, blood changes precede detectable alterations in the marrow, but signs of relapse occur in the marrow before they can be appreciated in the blood, they naturally take the form of megaloblastic increase at the

PLATE II



MEGALOBLASTS IN PERNICIOUS ANÆMIA

1 Proerythroblast and myelocyte
2 Basophil megaloblast
3 Hæmoglobinisation of megaloblasts
4 Abnormal megaloblasts
5 " "
6 " "

[To face p. 45

expense of the normoblasts Nevertheless, the most important purpose of marrow examination in this malady is the understanding of the morphological changes that characterise the disease, no other method has cast so much light into the dark places that surround the erythropoietic process as a whole

There are three other diseases in which an anæmia indistinguishable from pernicious anæmia may occur These are intestinal infestation with *diphyllobothrium latum*, *tropical nutritional anæmia*, due to deficiencies in the diet, and the *macrocytic anæmia of pregnancy* (see p 54)

The megalocytic anæmias associated with disease of the liver are of the same type as pernicious anæmia, but are rarely severe and will react to liver therapy If cirrhosis of the liver gives rise to much gastric hæmorrhage the marrow picture will be complicated by a normoblastosis and leucoblastosis as in the hæmorrhagic anæmias Anæmia associated with lesions of the alimentary canal is rarely due solely to lack of hæmopoietic principle

The conditions have been classified by Wilson as follows:—

(1) *Deficient intake of extrinsic factor*—tropical and nutritional macrocytic anæmia including the macrocytic anæmia of pellagra

(2) *Decreased formation of intrinsic factor*—gastric and intestinal resections and possibly carcinoma of the stomach

(3) *Impaired absorption of hæmopoietic principle*—sprue, cœliac disease, worm infestations, Crohn s disease and intestinal anastomoses and ulceration

(4) *Impaired storage and metabolism of the principle in the liver*—cirrhosis of the liver and possibly pernicious anæmia of pregnancy

(5) *Impaired utilisation of the principle in the liver*—achrestic anæmia

In all the conditions, anæmia may be due to lack of hæmopoietic principle or iron, or commonly a combination of both The most common anæmia with alimentary disease is certainly a hypochromic microcytic one associated with failure to absorb iron The myelogram will be fully discussed later In the combined deficiency, the marrow megaloblastosis is present with the changes due to lack of iron In these cases marrow puncture may give indications of great therapeutic value in showing that both iron and liver are necessary for adequate treatment Quite often the peripheral blood shows only the macrocytosis which is treated with liver, only when the hæmoglobin becomes constant at a relatively low figure with a low colour index does the need for iron become apparent Both thera-

peutic agents could have been given at once if the need for them had been recognised at first (see Dimorphic Anæmia, p 53)

Anæmia in Gastric Carcinoma Currie states that the marrow condition may be megaloblastic, as in pernicious anæmia , normoblastic, as in any ordinary hypochromic anæmia , and erythro blastic

The last named has not been clearly described before It appears that the marrow is very cellular and active, with many mitoses The predominant cells are macroblasts, *i e* , large, *basophilic normoblasts* They may be as large as megaloblasts, but have a definitely " cartwheel ' arrangement of the nuclear chromatin The anæmia in patients with this type of marrow is more severe than in those with a megaloblastic reaction

Currie is of the opinion that the type of marrow reaction is not related to the severity of the gastric lesion , and he also says that there is no obvious correlation between the apparent degree of deficiency of specific hæmopoietic substance and the type of reaction of the erythron ' Deficiency of specific hæmopoietic substance *per se* does not appear to result in a megaloblastic marrow reaction and a megalocytic anæmia In 59 cases examined by Monasterio the anæmia was of the normochromic normocytic type with many basophil normoblasts in the marrow

Achrestic Anæmia In this malady the marrow changes are similar to those seen in pernicious anæmia and the mixed megalo blastic and normoblastic picture seen in partly treated pernicious anæmia is often encountered Israels and Wilkinson find these observations to be in keeping with the view that in achrestic anæmia there is some inhibition of the action of hæmopoietin on erythropoiesis

Macrocytic Anæmia in other Conditions A variety of single cases have appeared in the literature in which the primary disease has been unexpectedly associated with a macrocytic anæmia and a megaloblastic bone marrow No reason is apparent for such associations, but it should be remembered that they may occur Those most recently recorded are generalised tuberculosis and macrocytic anæmia (Merwe), increased erythrocyte fragility associated with a macrocytic hæmolytic anæmia (Duke and Young), and an acute erythroblastic anæmia of childhood (McLean) Foy and Kondi have reported an extremely unusual case where a megaloblastic marrow was associated with a severe microcytic anæmia of the peripheral blood The patient was a pregnant Bechuanaland negress suffering

from amœbic dysentery and made a complete recovery after a course of emetine injections

Davis has reported three cases of megaloblastic anæmia in children aged respectively twelve, thirteen and fifteen years The blood and marrow pictures were characteristic of pernicious anæmia Two of the cases proved refractory to parenteral liver injections, but responded to proteolysed liver administered by mouth, and Davis suggests that these cases were due to failure of intestinal assimilation and not true examples of pernicious anæmia

Deficiency of Iron This results chiefly in the malady known as idiopathic hypochromic anæmia (Plate 10B) so clearly depicted by Witts, the nutritional anæmias associated with infancy, the anæmia associated with alimentary disease, and some anæmias of pregnancy Where the anæmia is solely due to deficiency of iron, the blood shows no spectacular changes and there are never very many normoblasts The marrow, however, is characterised by a great preponderance of normoblasts, many of which are large, immature, and strikingly basophilic The whole picture is one of very active erythropoiesis, and mitotic figures are numerous these are unlike those seen in the megaloblasts of pernicious anæmia, inasmuch as they are all typical abnormal karyokinetic figures are never seen

Neutrophiles	
Myelocytes	17–22 per cent
Metamyelocytes	5– 8 ,,
Polymorphs	8–12 , ,,
Eosinophiles	
Myelocytes	2– 4 ,, ,,
Polymorphs	2– 4 ,, ,,
Premyelocytes	1– 3 , ,,
Myeloblasts	0– 2 ,, ,,
Pro erythroblasts	8–10 ,, ,,
Normoblasts (basophilic)	20–30 ,, ,,
Normoblasts (eosinophilic)	20–30 ,, ,,
Megakaryocytes	scanty
Reticulocytes up to about 6 per cent of the non nucleated elements	

The activity of the myeloid tissue is somewhat reduced, with a consequent reduction in the number of myelocytes this in marked distinction to the microcytic hypochromic anæmia produced by

hæmorrhage The eosinophiles, however, remain normal in numbers or may show a slight increase

The myelogram has the characters shown above, which, of course, vary considerably as the result of treatment and as so many patients have been given some iron before they are properly investigated, characteristic pictures are uncommon

During, and even after, successful treatment with iron, the eosinophiles in the marrow increase considerably, although there may be no noteworthy *excess in the blood* *In pernicious anæmia*, as we have seen, eosinophilia may develop in the marrow and in the blood, but this is usually a sign of overdosage with liver (which is, of course, quite innocuous) but it is interesting to note that eosinophilia is a condition that develops during recovery from several kinds of anæmia

As in pernicious anæmia, one of the most striking features of the marrow during the phase of recovery is the increase in the number of reticulocytes These may increase to form 20–30 per cent of the *total non-nucleated elements* There *is, however, one difference* between the two diseases In pernicious anæmia the reticulocyte response is high if the red cell count is low, and low if the red cell count is high , in hypochromic microcytic anæmia the reticulocyte response depends more on the hæmoglobin level If this is moderately high, the response is poor, and *vice versa* In both maladies, the reticulocyte response to therapy is extremely rapid

Other changes in the marrow during the period of recovery are a gradual restoration of the normal number of neutrophiles and a steady *drop in the number of normoblasts* the percentage of basophil normoblasts drops more rapidly, till, when the anæmia is abolished, they and the eosinophil normoblast have reached a normal numerical relationship

The iron deficiency anæmias of pregnancy are similar to idiopathic hypochromic anæmia in blood and marrow The nutritional anæmias of infancy show the same changes in the peripheral blood, but here, of course, sternal *puncture is not possible*

Deficiency of Vitamin C Anæmia is not a constant finding in scurvy, but is commoner in adults than in children The anæmia is normochromic or hypochromic and is cured by the administration of vitamin C, whereas iron has no effect on it Ascorbic acid is a reducing agent and it is probably because of this that its absence, as in scurvy, leads to anæmia Normally it plays its part in the gut by reducing ferric compounds to the *ferrous state, thus making*

them suitable for absorption In the cases examined by Israels the sternal marrow showed evidence of diminished erythropoiesis, i.e, the total number of normoblasts was decreased, but their development was normal During treatment with vitamin C, reticulocytosis develops

If there has been much hæmorrhage into the tissues with the disease, the peripheral blood may show a microcytic anæmia The marrow, however, is unable to respond in the usual way, and the myelogram of hæmorrhagic anæmia does not develop until vitamin C has been given A few cases of macrocytic anæmia with scurvy have been reported, but the sternal marrow findings are not recorded

Deficiency of Thyroxin The exact relationship of thyroxin to the hæmopoietic tissues is not yet worked out, but it is probable that it acts as a general stimulant rather than on any particular hæmopoietic tissue

In myxœdema, if uncomplicated by deficiency of iron and of hæmopoietic principle, the bone-marrow shows simple hypoplasia In thyrotoxicosis there is a general hyperplasia of the bone marrow which may affect the myeloid elements to a greater degree than the erythroblastic tissue

Banti's Syndrome In the early stages of the malady, while the anæmia and leucopenia are not severe, the marrow films show a definite myeloid hyperplasia with a relatively normal ratio of myelocytes, metamyelocytes and neutrophils while no abnormality can be detected in the erythroblastic activity Later the marrow shows a " maturation arrest " of the hyperplastic myeloid tissue with an increase in the percentage of myelocytes while the neutropenia of the peripheral blood is becoming more severe At this stage thrombocytopenia may be well marked but the marrow appears to be well stocked with megakaryocytes In the later stages of the condition with severe macrocytic anæmia and cirrhosis of the liver the myeloid hyperplasia persists, but there is a marked increase in the number of immature normoblasts In Felty's syndrome (enlargement of spleen and glands, anæmia and chronic arthritis) the marrow changes are similar, but some authors (Limarzi *et al*, Fleischacker and Lachnit) have reported an increase in the number of hæmohistioblasts and plasma cells

Dimorphic Anæmia In this malady there is a deficiency of hæmopoietin and iron, and it may occur as a complication of al tary disease In those idiopathic cases which we have peripheral blood picture has been that of pernicious

the administration of liver extracts has been followed by an improvement in the anæmia with a more or less rapid lowering of the colour index to well below normal Further treatment with liver has no effect and the blood picture is only restored to normal after large quantities of iron have been given The appearances of the sternal marrow vary considerably Trowell has examined 174 cases of combined deficiency and says that the predominating type of erythropoiesis is normoblastic although megaloblasts and the large basophil normoblasts of iron deficiency can also be found In our experience the picture resembles closely that of a partly treated pernicious anæmia with an active normoblastosis and some megalo blastosis, the number of reticulocytes present, however, in the untreated disease is very small The condition can be distinguished from achrestic anæmia (the marrow changes of which are very similar) by the absence of free acid in the gastric juice Megalocytic anæmias associated with dietary deficiencies are often dimorphic in type

Anæmia in Pregnancy This may be normocytic, microcytic or macrocytic In normal pregnancy, hydræmia occurs and this is accompanied by a degree of normochromic normocytic anæmia of the peripheral blood and myeloid and megakaryocyte hyperplasia of the bone-marrow That these changes do accompany normal pregnancy is perhaps not generally recognised, and there is a need for the establishment of a set of normal standards during pregnancy

The true anæmias of pregnancy are mostly due to partial failure of production of hæmopoietic principle owing to temporary derangement of gastric function and the necessity of supplying the principle to the fœtus

Two types of macrocytic anæmia have been described by Markoff the first is a true pernicious anæmia of pregnancy and the second a macrocytic anæmia in which the marrow show both normoblastosis and megaloblastosis

Wolff and Limarzi claim that the type of anæmia in pregnancy should only be diagnosed from marrow examination because the changes in the peripheral blood may be misleading Apart from a typical iron deficiency anæmia, they have described a macrocytic anæmia with a normal or pro normoblastic marrow and a megaloblastic marrow with macrocytic, normocytic or microcytic and hypochromic anæmia, in the former type recovery follows delivery, and in the latter a good response can be obtained with liver therapy and the conditions disappears spontaneously in the puerperium.

In our experience, all these forms have occurred, but a small proportion of them, including some of those which present a picture of iron deficiency do not clear up after delivery and despite numerous types of therapy have continued to show a moderate degree of anæmia with persistence of the changes in the bone-marrow

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macroblasts or even pro-erythroblasts There is also some leucopoiesis in the liver, but this is not intimately mixed with the immature red cells

The sternal bone-marrow may appear normal during the greater part of the disease, only becoming involved in the terminal stage *It is important to remember this, for it shows very clearly that it is* never safe to assume that, because the marrow is normal, we are not dealing with a disease of the hæmopoietic tissues It has long been known that the blood may still appear healthy at a time when the formative organs are already abnormal, but it is less widely recognised that the bone-marrow may appear healthy at a time when the blood already shows signs of disorder

What little evidence is available seems to suggest that erythroleukæmia is a special mode of reaction to some unknown noxious [illegible]

Neutrophiles	
Myelocytes	10–18 per cent
Metamyelocytes	5– 7 " "
Polymorphs	35–42 " "
Eosinophiles	2– 4 " "
Lymphocytes	1– 2 " "
Monocytes	8–12 " "
Premyelocytes	0– 1 " "
Hæmocytoblasts	1– 3 " "
Normoblasts	12–18 " "
Megaloblasts	1– 3 " "

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CHAPTER VI

INFECTIVE DISEASES

LEUCOCYTE counts have been of considerable value in medicine and surgery as well as in more purely academic studies of immunity The blood changes in infective diseases are relatively well known, but the marrow findings have not yet been investigated in much detail

The types of changes that may occur can be classified as follows —

(1) Normal marrow with no alteration in the percentage com position

(2) Hyperplastic marrow with predominance of mature and almost mature neutrophiles

(3) Hyperplastic marrow with many immature neutrophiles, mainly myelocytes

(4) Extremely immature marrow in which premyelocytes are numerous or even prominent

(5) Myeloblastic marrow

An alternative classification suggested by Barta was —

(1) Moderate reaction with plentiful cells

(2) Intense reaction with immature cells including many myelocytes

(3) Extreme reaction with many premyelocytes or even myeloblasts, and

(4) Failure of reaction with decrease of granular leucocytes

No rigid classification is possible because there is a continuous series of gradations between the different types of marrow All that can profitably be done is to call attention to the outstanding characters of the myelogram

As in the blood so in the marrow, the picture is not pathognomonic of a particular infection The myelogram, like the hæmogram, gives an indication of the intensity of reaction but not of the under lying cause In a few infections it is alleged that there are pathognomonic changes, for example in both lobar and bronchopneumonia there is a greater degree of variation in the size of the granulocytes than in health, but this anisocytosis is not visible in the blood and

Normoblasts	25–25 per cent
Megaloblasts	1– 3 „ „
Megakaryocytes	scanty, but always present

Recently several authors have doubted whether there is, in fact, any true monocytic reaction in the bone-marrow There is no doubt that marrow smears in this disease often shows a marked *increase in monocytoid cells, but it is possible that this may be the* result of admixture with circulating blood and not a specific response of the marrow cells Perusal of the literature of bone-marrow biopsy in glandular fever indicates that about half of the reports show an increase in monocytic cells and half do not Limarzi, Paul and *Poucher give a good review of this subject and analyse twenty five* cases of their own Their conclusion is that the marrow does show a myeloid hyperplasia and immaturity but is not involved in the production of the monocytoid cells The average of the findings in the twenty-five cases is given as follows —

Myeloblasts	0 33 per cent	Range 0 – 2 25
Premyelocytes	0 82 „ „	„ 0 – 3 0
Myelocytes	20 0 „ „	„ 4 5 –48 0
Metamyelocytes	48 0 „ „	„ 24 0 –68 3
Polymorphs	5 55 „ „	„ 1 5 –19 5
Eosinophiles	0 78 „ „	„ 0 – 2 25
Basophils	0 06 „ „	„ 0 – 1 5
Proerythroblasts	1 70 „ „	„ 0 75– 7 5
Basophil Normoblasts	2 08 „ „	„ 0 5 –4 25
Polychrome „	21 8 „ „	„ 19 0 –24 0
Eosinophil „	0 5 „ „	„ 0 – 1 75
Monocytoid . .	2 5 „ „	„ 1 0 – 6 0

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CHAPTER VII

HYPOPLASIA AND APLASIA OF THE BONE-MARROW

APLASIA of the bone-marrow may arise from a variety of causes, ranging from exhaustion, to poisoning or to lack of specific nutritional factors A heterogenous group of diseases is involved including anæmia, agranulocytosis, and purpura, or a combination of all three, according to which part of the hæmopoietic tissue is involved Sternal puncture has been of great use in elucidating the pathology of these conditions and has shown that the peripheral blood may give a very inaccurate reflection of the conditions in the hæmopoietic bone marrow This tissue may be truly aplastic with failure of division of the primitive cells (primary aplasia), or there may be normal division of the precursor cells resulting in a very cellular marrow, which, owing to lack of some maturation factor, is unable to furnish finished elements to the blood stream (maturation type)

Whitby and Britton have made a useful classification of these diseases based on the marrow findings in the separate or combined affections of the erythroblastic, leucoblastic and thromboblastic tissues —

1 Aplasia of the erythroblastic tissue
(a) Primary—pure red cell anæmia The authors have found only five cases in the literature
(b) Maturation defect—dyshæmopoietic anæmias may become aplastic if the maturation factor is never supplied
2 Aplasia of the leucoblastic tissue
(a) Primary—agranulocytic angina
(b) Maturation defect—agranulocytic angina with marked myeloid reaction in the marrow These cases respond more readily to pentnucleotide therapy, which possibly supplies some maturation factor
3 Aplasia of the thromboblastic tissue
(a) Primary—essential thrombocytopenic purpura An acute form in which there is no rise of platelets after splenectomy
(b) Maturation defect—essential thrombocytopenia Splenectomy is probably beneficial in this type by removing a factor which inhibits platelet maturation

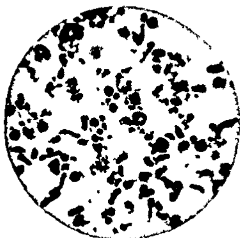
the predominant cells in most cases of this type of aplastic anæmia, and there is also a considerable excess of monocytoid elements, many of which are obviously phagocytic But it is always difficult, and sometimes impossible, to classify accurately lymphocytes, monocytes and pathologically altered myelocytes Davidson calls these unclassifiable elements "Q" cells If myeloblasts are present they are always abnormal, commonly they have indented nuclei, which may even be lobulated (Reider type)

In one case of idiopathic aplastic anæmia recently seen, the marrow showed some hypoplasia of all the nucleated elements (which were otherwise normal) and gross degenerative changes in the erythrocytes which assumed bizarre forms in the films and even appeared fragmented In the peripheral blood, however, the erythrocytes appeared normal except for a slight macrocytosis (Fig III)

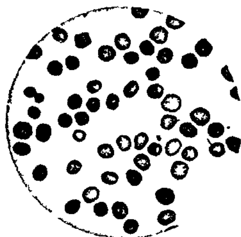
In aplastic anæmia with a hypercellular marrow the clinical picture is indistinguishable from that with a hypoplastic marrow We have during the last two years had under observation a girl of sixteen years whose peripheral blood shows a progressive normocytic anæmia with no change in the white cells The whole variety of hæmatinic drugs has no effect on her condition and every three or *four months she is transfused with 2 or 3 pints of blood* This raises her hæmoglobin to between 60 and 80 per cent Haldane, which level she will maintain and sometimes improve slightly for about a month It then gradually falls to between 30 and 40 per cent, at which level the transfusion is repeated Sternal puncture has always shown a hypercellular marrow with a normal differential count except of a slight persistent increase in the megaloblastic red cell series A characteristic count from this case is as follows :—

Hæmocytoblasts	0 5 per cent.
Myeloblasts	1 0 ,, ,,
Premyelocytes	1 0 ,, ,
Myelocytes	13 0 , ,,
Metamyelocytes	22 0 , ,,
Neutrophils	12 0 ,, ,,
Eosinophils	2 0 ,, ,
Basophils	0 5 ,, ,,
Pro-erythroblasts	0 5 ,, ,
Basophil megaloblasts	0 5 ,, ,,

FIG III



A



B

APLASTIC ANÆMIA

A Fragmentation of red cells in marrow

B Normal cells in peripheral blood

Polychromatic megaloblasts	1 5	per	cent
Eosinophils megaloblasts	1 5	,,	,,
Basophil normoblasts	5 5	,,	,,
Polychromatic normoblasts	6 0	,,	,,
Eosinophil normoblasts	13 5	,,	,,
Lymphocytes	15 0	,,	,,
Plasma cells	1 0	,,	,,
Unidentified cells	3 0	,,	,,

Any of the agents that can cause aplastic anæmia, *e g* , benzol arsenobenzene, thorium, the more modern sulpha compounds, X-rays, etc , may in smaller concentrations (or after less prolonged exposure) cause a less severe hypoplastic anæmia It is unfortunate that, although such stages are, at first at least, less grave, they tend to deteriorate, and recovery is very uncommon All these substances tend to attack the leucoblastic tissues first and the erythroblastic tissues later X-rays behave in the same way With sulphapyridine the effect on the leucoblastic tissue may be so severe that death ensues before any anæmia makes itself apparent, and these cases are often classed as agranulocytosis Benzol commonly attacks the leucoblastic tissue first, but cases have been recorded in which the platelets were first affected Even when there is a marked leucopenia, due to benzol, there may be some excess of eosinophiles both in the blood and in the marrow These changes also occur with drugs containing the benzol ring, *e g* , arsphenamine Six cases of blood dyscrasia with characteristic marrow changes after the exhibition of arsphenamine have been reported by Ferguson

It is important to realise that the myelogram can give us very *early* information in cases of suspected poisoning of the bone marrow with *these compounds* The number of people working with benzol has increased enormously during the years of the war, and we have had the opportunity of examining large numbers of these In some workers, showing the typical clinical syndrome of benzol poisoning, the hæmatological findings have proved negative Sternal puncture, however, may show definite alteration in the hæmopoietic tissue The myelogram quoted below was found in a female benzol worker, aged thirty-three, who had complained of dizziness, lassitude and menorrhagia for some four weeks Her blood count showed a moderate microcytic hypochromic anæmia (Hb 68 per cent , R B C 4,000,000, C I 0 85) The white blood count was 5,500 per cubic millimetre and the differential —polymorphs 61 per cent , lympho-

cytes 32 per cent, monocytes 4 per cent, eosinophiles 3 per cent The myelogram was as follows —

Neutrophiles	
Myelocytes	14 per cent
Metamyelocytes	10 " "
Polymorphs	23 " "
Eosinophiles	
Myelocytes	1 5 , ,
Polymorphs	1 0 " "
Lymphocytes	26 5 , "
Monocytes	4 5 "
Myeloblasts	2 5 " "
Normoblasts	17 " ,
Megakaryocytes	very scanty

Reticulocytes 1 5 per cent of the non nucleated elements

Here we see at once some depression *in the myeloid tissue* Normoblasts are present in normal numbers, but not in such large quantities as one would expect if the anæmia were due to iron deficiency The low reticulocyte count is striking The number of lymphocytes is raised, and this point brings one to an interesting speculation We know that it is not possible at the moment to determine the activity of the bone marrow by doing cell counts on *the sternal marrow fluid, but we may assume that if there is no* absolute lymphocytosis or lymphopenia in the peripheral blood, an increase in the percentage of lymphocytes in the bone marrow is only a relative increase, and that there is no alteration in their absolute number Then, an increase in the percentage of lymphocytes can be used as an index of the decrease in the amount of the other elements of the marrow In the case quoted above, the absolute number of lymphocytes in the *peripheral blood is within* normal limits (1,820), in the marrow, however, the lymphocytes *are increased one* and a *half to two times* It would therefore seem reasonable to assume that the myeloid and erythroblastic elements are reduced to the same extent, as there is no valid reason to account for an absolute increase of lymphocytes in the bone marrow Whether *this inference is admissible or not, the other observation that this* marrow showed a general depression compatible with benzol poisoning, before any typical changes in the peripheral blood were *noted, is of diagnostic value*

When anæmia due to benzol poisoning is fully established well-

marked changes are always present in the bone marrow Mallory, Gall and Brickley have investigated a series of cases and found that between 30 and 40 per cent showed severe hypoplasia of the sternal marrow (no cases of complete aplasia were seen), about 40 per cent showed normal cellularity and about 20 per cent showed a hyperplastic condition In the hypoplastic type the myelogram was substantially the same as that already quoted (p 68), and one case showed a high proportion of plasma cells, some with multiple nuclei In the normocellular group there was an increase in the proportion of granulocytes and megakaryocytes with what the authors call numerous " clumps of hæmocytoblasts " In the hyperplastic type proliferative activity was sometimes extreme with not infrequent multipolar mitoses, the hæmocytoblasts and megakaryocytes were also increased in number, the latter showing abnormal forms Erf and Rhoads have reported similar findings and refer to the increased number of " primitive " cells from 4 to 34 per cent It must be remembered that leukæmia may develop after benzol poisoning and the marrow then shows the changes ordinarily seen in leukæmia Mallory *et al* and Erf and Rhoads quote typical cases in their papers

One further case may be quoted where changes in the sternal marrow were detected before they appeared in the blood A male,

polymorphonuclears were seen in the film Sternal puncture was

blasts numbered only 12 per cent The lymphocytes were 25 per cent The polymorphs were very degenerate, and there were only 10 per cent of myelocytes Here we had a primary aplasia of both the leucoblastic and erythroblastic tissue, the latter not yet being apparent in the blood Two days later the hæmoglobin and the red cell counts began to fall and the patient died in spite of repeated transfusions with fresh blood

One more point remains to be mentioned in connection with this group of marrow poisons It would seem that only susceptible persons are affected Most cases of fatal poisoning with benzol have

large numbers (perhaps normal numbers) of the less mature forms of granulocytes, but practically no polymorphonuclears, and the peripheral blood shows the typical picture of extreme leucopenia, with *relative lymphocytosis* Of the granulocyte precursors, Jaffe has found that the myeloblasts show no morphological abnormality, but the myelocytes show degenerative changes in the nucleus *which eventually lead to the death of the cell (Plate 12A)* The nuclear changes are preceded by some splitting and degeneration of the cytoplasmic granules Jaffe emphasises these changes as the specific pathological features of the malady, and *it would seem that* they depend upon the absence of some specific factor that is normally required for the proper differentiation of the myelocytes if this [illegible] would be good The available [illegible] substances as sodium pentose nucleotide is often followed by cure But in the aplastic form, there is little evidence that treatment on these lines is of any value at all The myelogram in the " maturation ' type of agranulocytosis is of the following type —

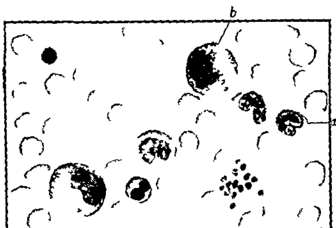
Neutrophiles	
Myelocytes	30–35 per cent
Polymorphs	1– 5 " "
Eosinophiles	
Myelocytes	1– 2 "
Premyelocytes	1– 2 , "
Hæmocytoblasts (including myeloblasts)	1– 2 , "
Lymphocytes	20–35 " "
Normoblasts	15–20 , "
Megakaryocytes	scanty

The myelocytes show degenerative changes in the nucleus and specific cytoplasmic granules

Whether the aplastic type of agranulocytosis is an advanced stage of the " maturation ' type is still unknown , but it is obvious that, in spite of this large gap in our knowledge, sternal puncture is of the utmost prognostic value By it we can determine whether there is any hope that treatment will do good, or whether, with the means available, death is inevitable

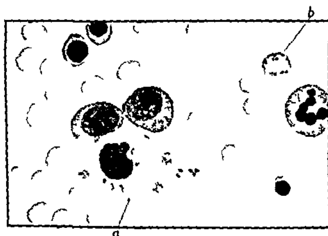
Essential Thrombocytopenia *In this malady,* as with agranulocytosis, there would seem to be a primary aplastic form as well as a ' maturation " type resulting from failure of the mega-

PLATE 12



A AGRA LOCYTOS S (MAT RAT ON TYPE

No e Abs n e of p morphs non granula ty of me am eloc es (*a*) nu lea degen on n myeloc es (*b*



B THRO BOCYTOPE A (M TUR T ON TYPE)

No e Abnormal megakaryocyte (*a*) and giant p a elet (*b*)

karyocytes to develop properly Clinically the aplastic form is acute illness and splenectomy is attended by a 70–80 per c mortality In the chronic recurring type or phase of the malady marrow often shows a maturation defect, and in these cases splen tomy practically always alleviates the condition This has led to supposition that the spleen in these cases produces an inhibi factor which acts on the bone-marrow

In the primary aplastic form of the disease, the significant cha is the complete absence of megakaryocytes and the paucity of pl lets Careful search of even the ends of the films fails to reveal fragments of megakaryocytes and such platelets as there are may deformed If any anæmia has resulted from extensive purp bleeding the myelogram will show a normoblastic hyperplasia, this is to be regarded as a reaction to the loss of blood and not part of the malady itself

In the " maturation " type, megakaryocytes are present in nor numbers and show some alteration in structure Platelets deficient, however in number and quality, giant forms are m more common than in normal marrow (Plate 12B) The m karyocytes, although as large as fully differentiated forms pre much less nuclear lobulation and contortion The cytoplasn often vacuolated and may be completely free from granules, whether this is a sign of degeneration, as some writers have c tended, is quite unknown The one point that suggests the m karyocytes are, indeed, the seat of degenerative changes, is that number of platelets, both in the marrow and in the blood, is gre reduced, and it is reasonable to suppose that this may be due to of functional activity of the parent forms

Dameshek and Miller have counted the number of megakaryoc in bone-marrow biopsies in idiopathic thrombocytopenic purp normals, and in cases of thrombocytopenic purpura associated various types of splenomegaly Their figures are as follows -

1 Normals—not more than 300 megakaryocytes per mil nucleated red cells, 69 per cent showing platelet product
2 Chronic idiopathic thrombocytopenic purpura—increased n ber of megakaryocytes with decreased platelet productio
3 Splenomegaly of non leukæmic origin (cirrhosis, Gauch disease, etc) slightly *increased megakaryocytes* with no platelet production
4 Aplastic anæmia, lymphosarcoma, acute leukæmia—g

reduction of megakaryocytes a normal percentage of which show platelet production

Although a hypoplastic or aplastic condition of the bone marrow is not involved, other forms of purpura may well be mentioned here Sternal puncture is valuable because there are so many conditions that can give rise to purpura, and their accurate differentiation is of great therapeutic importance Thus we may find a myeloblastic overgrowth of the marrow which demonstrates that we are dealing with a leukæmia, or, we may discover a marrow that is entirely *devoid of erythroblastic and granulocytic elements*, and we realise that the malady is some form of aplastic anæmia

In the purpuras associated with infection or with the action of poisons, there may be a transient increase in the number of megakaryocytes, but quite soon these elements become scanty Moeschlin, however, could find no changes in the bone marrow of people who developed thrombocytopenia after the administration of sedormid The myelogram is of the following type —

Neutrophiles	
Myelocytes	10–15 per cent
Metamyelocytes	4– 6 ,, ,,
Polymorphs	18–25
Eosinophile polymorphs	2– 4 ,, ,,
Premyelocytes	2– 4 ,, ,,
Lymphocytes	1– 2 ,, ,,
Monocytes	1– 3 ,, ,,
Plasma cells	1– 3 ,, ,,
Pro erythroblasts	3– 5 ,,
Normoblasts	30–45 ,,
Megaloblasts	2– 4 ,, ,,
Megakaryocytes	1– 4 ,, ,,

There seem to be several conditions in which the condition of the peripheral blood suggests that there is hypoplasia of one or all the components of the myeloid tissue, but in which marrow puncture reveals intense hyperplasia Thus, excessive thrombolysis in the spleen can produce the picture of thrombocytopenic purpura, although there is increased formation of platelets in the marrow And Doan and Wright have described a condition of *splenic panhæmatopenia* in which every type of marrow cell is increased in number, although the blood picture suggests the opposite of this

They adduce evidence that, in this condition, there is indiscriminate destruction of every kind of formed hæmic element as it passes through the spleen.

Cases which are clinically indistinguishable from essential thrombocytopenia may occur as "allergic" manifestations, particularly after streptococcal infections Schwartz has suggested that examination of marrow smears may differentiate this condition from the idiopathic type because in the former there is an increased number of eosinophils This eosinophilia of the marrow in the infective type is not necessarily reflected in the peripheral blood

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SOME PROTOZOAL DISEASES

It would seem that with these diseases there is an extensive and promising field of work in connection with sternal puncture, as many of the parasites make their home in the reticulo-endothelial system, and so one would expect to find them frequently in the bone marrow. Also the literature on the subject is scanty. The enlargement and softening of the spleen, which so often accompany these diseases, may make spleen puncture an operation of some consequence because of the possibility of persistent bleeding. Liver puncture, though safe, does not usually give very satisfactory material for examination. Sternal puncture, however, is rapid, safe, and provides material which not only may contain the parasites in large numbers, but at the same time provides an opportunity of explaining the more or less characteristic blood changes.

Kala Azar (Plate 13A). Let it not be supposed that this disease is confined to the tropics; many cases from the margins of the Mediterranean basin and from the Sudan have been recorded, and one is tempted to presume that even more have been overlooked.

The myelogram has remarkable characters, inasmuch as there is lymphocytosis, increase of monocytes, intense reticulo-endothelial hyperplasia, patchy deposition of platelets and, of course, the pathogenic agent, the Leishmania itself. The last is found lying inside monocytes, but some lie free and rather closely resemble platelets, with which they may easily be confused. Close examination shows [illegible] a more complicated structure than have

[illegible]

is probably an artefact due to rupture of the cells during spreading of the film. In the films we have seen, where a group of Leishmania is encountered, they are always entangled in a faintly basophilic web, probably representing the remains of the monocyte cytoplasm; quite often a degenerate nucleus is found in close proximity. Extracellular forms must occur in the passage from cell to cell, but these are rare and probably occur singly. In histological preparations, where the cells remain intact, the parasites are almost invariably intracellular. In films also they may sometimes be seen superimposed on a red cell, and they have been thought to lie within them, as with the malarial parasite. This is an artefact. The organisms may be found in peripheral blood films but always within either the monocytes or the polymorphonuclears.

In man the parasite is in a non-flagellate stage It is circular, oval, or cigar-shaped in outline and is 2 to 4 microns long and 1 to 3 microns wide The cytoplasm, faintly basophilic, is contained in a tenuous membrane and contains two characteristic structures, which are essential to its recognition The one, the nucleus, is larger than the other, and is usually spherical Its diameter is about one-third to one-half of the shortest diameter of the organism It usually lies against the membrane and is flattened on this side The flattening may be extreme so that the nucleus appears as a thick line, and this appearance may be accentuated by the presence of vacuoles within the cytoplasm With Jenner-Giemsa stain the nucleus appears as a bright red granular mass. The second, the blepharoblast, is a short rod-shaped structure with one end pointing to the nucleus It may appear as a dot if its long axis is perpendicular to the slide The blepharoblast stains purple with Jenner Giemsa (Plate 13A)

Quite apart from the presence of Leishmania, the myelogram is characteristic, and may remain so for many months after suitable treatment has caused the disappearance of these organisms

Neutrophiles	
Myelocytes	18–24 per cent
Metamyelocytes	0– 1 ,, ,,
Polymorphs	8–10 ,, ,,
Eosinophiles	0– 1 ,, ,,
Lymphocytes	
Small	18–22 ,, ,,
Large	18–22 ,, ,,
Monocytes	14–18 ,, ,,
Plasma cells	1– 3 ,, ,,
Hæmohistioblasts	0– 1 ,, ,,
Hæmocytoblasts	0– 1 ,, ,,
Normoblasts	3– 5 ,, ,,
Megaloblasts	1– 3 ,, ,,

In Leishmania tropica infections (oriental sore) the lesions are usually confined to the skin, sometimes appearing in the mouth also Enlargement of the glands may occur if lymphadenitis is present and the organism has been found in them Very rarely it has been demonstrated in the peripheral blood, but further investigation may show it to occur more frequently in the bone-marrow

Malaria (Plate 13B) In benign tertian and quartan infections the

asexual life-cycle of the parasite takes place in the peripheral blood In malignant tertian fever, the cycle takes place in the tissues It would appear, from sternal puncture films of a case of cerebral malignant tertian malaria, that this schizogony occurs, in part at least, in the bone-marrow All the changes from the signet-ring stage to the rosette stage could be traced, while peripheral blood films showed only a few signet-ring and gametocyte forms The number of infected corpuscles in the marrow exceeded by far those in the bloodstream Plate 13B was prepared from this film

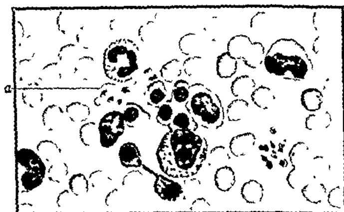
Recently several papers have come from the Middle East showing that sternal puncture may be of great value in the diagnosis of malaria Aitken examined 95 cases in which serial thick drop preparations made from the peripheral blood at six-hourly intervals had proved negative In 39 of these cases the parasites were found in the sternal marrow In a further series of 10 cases which did show the parasites in the peripheral blood, parasite-containing red cells were from 2 to 5 times more numerous in the marrow fluid Rumball and his co workers point out that most cases of pyrexial malaria can be diagnosed from the peripheral blood, but that sternal puncture forms a valuable supplementary diagnostic method in those cases where sporulation is scanty and parasites cannot be found in the peripheral blood

In response to the hæmolysis of red corpuscles by the malarial parasite, the marrow response is *essentially normoblastic and monocytic*—changes that do not disappear for many months after recovery Monocytosis, which is considerable in chronic cases, is less marked in acute ones *The following myelogram is of a fairly characteristic type*

Neutrophiles	
Myelocytes	5– 7 per cent
Metamyelocytes	0– 2 ,, ,,
Polymorphs	9–12 ,, ,,
Eosinophiles	0– 2 ,, ,,
Monocytes	10–14 ,, ,,
Premyelocytes	0– 2 ,, ,,
Hæmocytoblasts	0– 1 ,, ,,
Normoblasts	40–50 ,, ,,
Macronormoblasts	12–8 ,, ,,

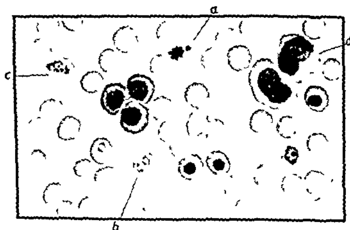
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A KALA AZAR

(a) Leishmania in ruptured monocyte



B MALARIA (MALIGNANT TERTIAN)

(*a*) Schizonts
(*b*) 2 ring forms in 1 corpuscle
(*c*) Young gametocyte
(*d*) Monocytes

[To face p. 98

THE TECHNIQUE OF STERNAL PUNCTURE

Anatomy. The cartilage of the second rib joins the side of the sternum at the junction of the manubrium and the body This junction can be felt, and often seen, as a transverse ridge on the front of the chest, as the two bones are slightly thickened at their point of union The ridge can usually be felt, in the adult male, about 2 inches below the jugular notch In the female, the manubrium is longer and the ridge consequently lower Until late adult life the

surface of the manubrium which adjoins the body of the sternum

The centre of ossification in the manubrium appears during the fifth month of fœtal life, and at three years of age the bone contains a moderate amount of red marrow Despite this, sternal puncture is an unsatisfactory procedure if performed much before the age of seven to eight years The needle enters the manubrium very easily but it is difficult to obtain any marrow at all Kato, however, prefers to puncture the body of the sternum, which, he states, contains cellular marrow even in infants of one or two years of age Even so, it is better, in the rare cases in which marrow examination in an infant is necessary, to obtain the specimen by trephining the tibia

Technique of Sternal Puncture This is a comparatively simple procedure, but in order to cause as little pain as possible, a few details must be adhered to

The type of needle to be used is of some importance, because it needs to be thick and strong, so as neither to bend nor break in piercing the bone Something in the nature of a very short French lumbar puncture needle, with an obturator stilette, is best, and a movable guard on the needle itself is useful to avoid the danger,

suction it is well to use a large syringe, *e g*, about 20 or 25 c c

It is probably not of much importance where the bone is punc-

tured, but as has been said, the thinnest spot lies on the under (caudal) surface of the manubrium—*that is in the upper surface of the joint* that forms the angle of Luis And this is the easiest and least painful site for the purpose



STERNAL PUNCTURE NEEDLE (WITTS TYPE)

The needle resembles a short thick lumbar puncture needle with obturating stilette and movable guard The guard is *so adjusted that there is no* possibility of piercing the posterior wall of the sternum If however it is found that the guard has been placed so near to the point that the *anterior wall is not pierced* it can be moved *after the needle* is in the tissues

Now the procedure is as follows the patient is placed on his back, with a pillow under the shoulders, so as to *throw the head backwards* Then novocaine is injected into the site of election, taking care that the deeper tissues, *including* the sternal perio steum, are infiltrated

The needle is plunged in just below *the angle of Luis, and when the point* is felt to reach this it is pushed a little further until it penetrates the small piece of cartilage in the joint When it seems to be in about the middle of this the butt of the needle is lowered until *it is almost touching the chest*, and then it is pushed straight upwards until the bone is pierced The manœuvre *is sometimes* accomplished more easily if a rotatory motion is imparted to the needle as in using a bradawl The sensation obtained when the outer layer of compact bone is penetrated is rather like that of a *lumbar puncture* when the point of the needle enters the theca Now the stilette is withdrawn and the *needle* pushed onwards, thus collecting marrow tissue in the lumen

Another method in common use is as follows The guard of the needle is set just above the point of the *needle*, which is *pushed* vertically against the anterior plate of the manubrium Then, when the resistance of the bone is felt, the guard on *the needle is set about 5 mm* above the skin surface, the needle is then pushed onwards If no marrow is obtained the

guard may be raised another 2 mm and the needle again pushed onwards, but the needle must never be pushed so far as to touch the posterior lamella of the sternum As the thickness of the cancellous bone is between 0 5 and 1 5 cm (Arjeff), there is no danger of penetrating the sternum by this method

A good deal of information can be obtained, after some experience, by the different sensations obtained as the needle moves onwards Thus the hard push needed in osteosclerosis is very different from that required by the soft marrow of a leukæmia

The syringe, either thoroughly dry or rinsed out with normal saline, is now attached to the needle and the plunger pulled out However well the tissues have been anæsthetised, there is now always an unpleasant sensation, which some patients allege amounts to pain, but it is quite transient Thick blood like material should enter the barrel of the syringe, but some marrows are too dense for this to occur even then a sausage of myeloid tissue will usually be found, often mixed with spicules or cancellous bone, lying in the lumen of the needle Suction is now stopped, and after removing the needle the puncture wound is sealed with collodion

It is best to expel the whole of the content [illegible]

[illegible]

it is impossible to find any fragments, and then films must be made from the thick fluid This is often difficult, as all but the most hyperplastic marrows contain globules of fat, which render the preparation of really good films difficult, and, of course, the spicules of cancellous bone make it even more so For this reason a large number of films should be prepared, and among them good ones will be found The films should be made as quickly as possible because marrow clots as rapidly as blood, but no attempt should be made to make the films as thin as those of blood, if this is tried, far too many cells will be ruptured in the attempt It is often possible to make thinner films by mixing some of the marrow juice with a little serum As the cells in thin films are further apart, this procedure simplifies the performance of the differential count

Turkel and Bethell have introduced a small trephine with which marrow can be obtained from the sternum with no more trouble than by the usual aspiration method. As material for histological examination can be obtained by this means, it is specially useful

in cases of myelosclerosis (p 19) In most other cases, aspiration is probably the method of choice

A warning must be given here about the use of post mortem specimens of marrow fluid Normally, gross degenerative changes have taken place in the cells three hours after death and the nuclei of all the cells stain a homogenous purplish blue colour with no visible chromatin pattern, the cytoplasm has often disintegrated completely so that bare nuclei, some surrounded by granules can be seen

The sternum is not the only bone from which marrow can be obtained by puncture Loge has called attention to the ease with which marrow can be obtained from the spinous processes of the third and fourth lumbar vertebræ The technique is much the same as that of sternal puncture, but the guard is set at 1 5 cm then, if no marrow is obtained by suction at this depth, it is safe to push on to a maximum of 2 5 cm

Loge found that the myelograms from sternal and spinous marrow are practically identical, and the same is true of marrow obtained by puncture of the iliac crest Clearly, this knowledge is of great value when serial punctures have to be performed on the same patient

Ordinary films can be stained by whatever method is desired, but as a rule it is, of course, sensible to use the same method as is used for blood, in this way comparison is considerably facilitated On the whole, Jenner s solution is not satisfactory, because the nuclear detail is poorly displayed, and because any azurophilic granules remain unstained Leishman's solution or the excellent Jenner-Giemsa method is very suitable In combined staining May-Grunwald may be used instead of Jenner, and Panchrom stain or Kardos mixture in place of Giemsa Different effects can be obtained by using different combinations, for instance, May Grunwald and Kardos mixture gives extremely brilliant granule staining

Vital Staining (a) Reticulocytes A clean slide is flooded with an alcoholic solution of cresyl blue This film is allowed to dry, and any tendency to roughness on the surface of the dye film is smoothed by gently polishing with a piece of soft paper One small drop of marrow fluid is put on the slide and gently stirred with a glass rod, taking care not to spread the marrow over too large an area so that drying takes place As the marrow fluid mixed with cresyl blue does not clot for some time, it is allowed to stand for

two or three minutes so that maximum staining of the reticulum is obtained. Films are then made from this drop and allowed to dry in air. They can be examined directly for reticulocytes or counterstained for making permanent preparations. Counterstaining, however, tends to hide the reticulation in cells which have only a little of the vitally stainable basophilic substance.

(b) Fresh preparations. The most commonly used dyes for this purpose are neutral red chloride and Janus green. The preparations are made in the usual way. Equally good results, however, can be obtained by dark ground illumination of fresh preparations as described below.

Dark Ground Illumination (Fig. IV). A good account of this technique was given by Whitby and Hynes. Thoroughly clean and degreased slides and coverslips must be used. A small drop of marrow fluid is placed on a slide and covered with a cover slip; the preparation is immediately sealed with wax and kept warm in the incubator. The slides should be covered so that light is excluded. If good results are to be obtained, the marrow films must be as thin as possible; the smaller the drop of marrow, the thinner the final preparation and, as a rule, the drop should be of such a size that when the cover slip has settled, the film does not quite reach its edges. In this way one attains the maximum capillary attraction between the slide and the cover, whereas if there is enough marrow fluid to reach the edges of the coverslip, this tends to float up and make a thick preparation.

After half an hour in the incubator, the preparation is examined with dark ground illumination and, if possible, some form of warm stage. Under proper conditions the movements of the motile cells are easily seen, as are also the mitochondria and other elements seen in any vitally stained preparations. Dark ground illumination has further advantages in that the cell and nuclear membranes are easily visible and that nuclear structure is also quite clear. The motility of cells is rapidly slowed down by light, so the preparation should only be illuminated for a few minutes at a time, and then allowed to recover in the dark. For observing cell structure the films last quite well for four to six hours without any protection or warming.

The characters of the cells are as follows (see Fig. IV).

(1) GRANULOCYTE SERIES. (A) *Myeloblast*. A rounded cell 8 to 10 microns in diameter. Thin defined cytoplasmic rim; nuclear membrane equally well defined and somewhat thicker. Nucleus rounded and appears filled with a fine dust; nucleoli appear as

circular outlines where the dust like particles are thicker Cytoplasm contains mitochondria which appear as faint dots Cell non motile

(B) *Premyelocyte* Ten to 20 microns in diameter and rounded, cytoplasmic rim, nuclear membrane, and nucleus as in the myeloblast The mitochrondria are now grouped round the nucleus and appear as thin, wavy rods, sometimes showing Brownian movement Cell non motile

(C and D) *Myelocyte* Ten to 20 microns in diameter The specific granules *appear in the cytoplasm of the premyelocyte in* increasing numbers first among the mitochondria Eventually they *fill the whole of the* cytoplasmic space and may partly obliterate the nucleus which has now no nucleolar structures The specific granules of each of the neutrophile, eosinophile, and basophile myelocytes are described under the mature polymorphonuclear cell

(E) *Neutrophile Polymorphonuclear* An active motile cell, 12 to 20 microns in diameter when at rest The nucleus, now lobed has
The cytoplasm is filled with
, the leading end of each
of granules

(F) *Eosinophile Polymorphonuclear* Less actively motile than the *neutrophile cell* and of about the same size The granules are readily distinguished as they are larger, appear as rings of light, and *may be oval*

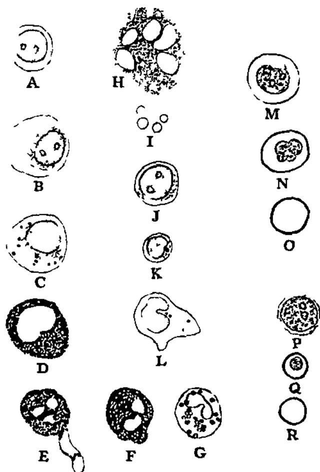
(G) *Basophile Polymorphonuclear* A smaller cell 7 to 10 microns in diameter *The granules are of the same type* as the eosinophile granules, but are much fewer in number and may appear even larger

(2) LYMPHOID SERIES (J) *Lymphoblast* A rounded cell, 8 to 10 microns in diameter The nucleus is *indistinguishable from that* of the myeloblast The two cells however, are easily separated by the appearances of the mitochondria *These in the lymphoblast* are larger, less numerous, and definitely rod shaped Cell non-motile

(K) *Lymphocyte* Rounded, may occasionally be motile and vary in size from 10 to 15 microns The nucleus is round or indented and filled with fine dust like granules The mitochondria are grouped to one side of the nucleus and appear as fine round *oval* dots Two three four or five large bright granules are present " Gall s mahogany granule " shows as a refractile ring of light

(3) THROMBOBLASTIC SERIES (H) *Megakaryocyte* A large cell up

FIG. IV



DARK GROUND ILLUMINATION OF MARROW CELLS

(a) Myeloblast
(b) Premyelocyte.
(c) (d) Myelocyte
(e) Neutrophile
(f) Eosinophile
(g) Basophile
(h) Megakaryocyte
(i) Platelets
(j) Lymphoblast
(k) Lymphocyte
(l) Monocyte
(m) Promegaloblast
(n) Megaloblast
(o) Megalocyte
(p) Early normobl
(q) Late normob
(r) Normocyte

Fc see p 74

to 40 microns in diameter. The nucleus usually appears as a group of separate lobes, each of which has a definite nuclear membrane, and is filled with fine dust-like particles The cytoplasm has no definite rim and is filled with small moderately bright granules

(I) *Platelets* These appear most as small circles of light, 2 to 5 microns in diameter The larger number appear to be empty, but some contain five or six small bright granules, which show active Brownian movement, apparently bouncing from side to side of the cell

(4) ERYTHROBLASTIC SERIES (M) *Promegaloblast* A non-motile cell about 10 microns in diameter The cytoplasmic rim is thick and well defined This cell shows marked pleomorphism The cytoplasm appears completely free of granules and other structures although there may be a group of small rounded mitochondria adherent to one side of the nucleus, when this occurs, the nuclear membrane is absent at the point of contact This membrane is otherwise well defined and thin The nucleus is filled with fine dust-like particles which are more closely packed and appear much brighter than those of the leucoblastic cells Also the nuclear particles show some small areas of condensation which appear as brighter granules The nucleoli, two or three in number, are outlined by a ring of such condensation

(N) *Megaloblast* Very variable in size and shape There is a thick cytoplasmic ring and clear cytoplasm without mitochondria The nucleus may appear lobed and shows areas of localised condensation

(P) *Early Normoblast* The nuclear structure is the same as in the megaloblast, and the nucleus is of much the same size The cytoplasmic ring is not well marked, and the cytoplasm is scanty and may contain a few dust-like mitochondria The cell is easily

a

(R) *Normocyte* This appears as a bright ring of light with a thick cytoplasmic rim The megalocyte (O), though larger, has the same appearance

Cell Propagation. Mitosis. While mitotic figures are easily recognisable, it must be remembered that the whole process is a continuous one, and that the early changes in the nucleus, between the interphase and the prophase, are very easily overlooked These changes may completely alter the apparent structure of the nucleus

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(Q) *Late Normoblast* Seven to 8 microns in diameter with a thick refractile rim Th–

(R) *Normocyte*

thick cytoplasmic ri

same appearance

Cell Propagation **Mitosis** While mitotic figures are easily recognisable it must be remembered that the whole process is a continuous one and that the early changes in the nucleus between the interphase and the prophase are very easily overlooked These changes may completely alter the apparent structure of the nucleus

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(R) *Normocyte* [illegible]
thick cytoplasmic ri[illegible]
same appearance

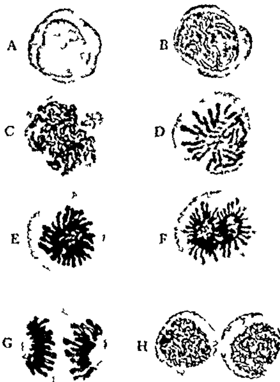
Cell Propagation. Mitosis While mitotic figures are easily recognisable, it must be remembered that the whole process is a continuous one, and that the early changes in the nucleus, between the interphase and the prophase, are very easily overlooked These changes may completely alter the apparent structure of the nucleus

and cause some confusion as to the actual nature of the cell The same remarks apply to the final transition from the telophase to the interphase, except that the presence of two daughter cells, lying in apposition, should give a clue to the real condition Failure to appreciate these changes, especially in histological material where there is much cell shrinkage, has led to much of the confusion which has existed over the morphological characters of blood cells and their apparent relationships

The advent of sternal puncture has made it possible to study these changes much more closely, and they are illustrated on Plate 14 The cells depicted here are promonocytes from a case of monocytic leukæmia Care has been taken to choose typical figures such as will be seen in normal hæmopoiesis One of the characteristics of a leukæmia is, of course, the presence of atypical mitoses, tri- and even tetrapolar figures can be seen

In the resting cell (A, Plate 14) the nucleus is rounded and the chromatin shows a fine, rather irregular reticulation The cytoplasm has a ground glass appearance In the earliest stage of the prophase the pattern of the nucleus becomes more distinct owing to the disappearance of small anastomosing links, leaving the permanent chromatic elements of the chromosomes, the chromonema (B, Plate 14) It is at this stage that the greatest care is required in labelling a cell, a lymphoblast in this stage of development might easily be mistaken for a myeloblast Some authors have described such changes in monocytes, and used them to suggest that the cell was derived from a lymphocyte In the later stage of the prophase (C, Plate 14) the chromosomes are beginning to appear as separate entities Each consists of two chromonemata lying parallel and fixed in an achromatic matrix During the metaphase this matrix stains intensely and the internal structure of the chromosome is no longer visible The arrangement of the chromosomes in the metaphase appears to be constant for all the cells of the hæmopoietic tissue The long chromosomes are arranged radially at the periphery, and the short ones lie irregularly in the centre (E, Plate 14) In the anaphase, the chromonema from each chromosome move to opposite poles so that each daughter nucleus will have a representative part of the parent chromosome The anaphase is shown in Fig F, Plate 14 In the telophase the matrix of each daughter nucleus becomes achromatic again and the chromosome structure is again visible (G, Plate 14) Further changes in the new nuclei are the same as in the prophase, only occurring in the reverse order (H, Plate 14)

PLATE 14



MITOTIC DIVISION (× 2,000)

(*See* p. 85)

The cytoplasm divides by furrowing It undergoes quite definite changes during the nuclear division, changing from a fine homogeneous structure to a mass of fine interwoven threads (Plate 14). The cytoplasm of dividing erythroblasts and normoblasts appears to be granular rather than thread-like.

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INDEX

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